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THE DOUBLE CHROMOSOME REPRODUCTION IN SPINACIA AND ITS CAUSES

II. AN X-RAY EXPERIMENT

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X-RAYS AND CHROMOSOME REPRODUCTION.

AFTER X-raying normal mitotic tissue three different effects are produced, named primary effect, mitose-free period effect and secondary effect (POLITZER, 1934; MARQUARDT, 1937 a, b, 1938). The primary effect appears after the treatment of dividing cells and consists of agglutination, pseudo-amitosis and fragmentation phenomena. Besides, actually dividing cells, when treated with X-rays, poisons, etc., are arrested at the metaphase stage for a certain time (delayed splitting of the centromeres?), but sooner or later the anaphase movement starts and the nuclei enter telophase and resting stage again. As POLITZER has shown, after the treatment of *Salamandra* larvae with killing dosages of alcohol, dividing cells continue mitosis even when no resting nuclei are able to divide.

The mitose-free period increases in duration as the X-ray dosage increases. Cells already in prophase continue division, but then follows a period of quiescence. The division-free period is highly pronounced in vegetative tissue (growing roots etc.), but is almost negligeable in meiosis and pollen-grain mitosis (MARQUARDT, 1938; SAX, 1938). Prophase start and time of chromosome splitting are simultaneous in germinating seeds and root mitosis but differ in pollen-grains, where chromosome splitting in a physiological sense takes place somewhere during the resting stage. The occurrence of a mitose-free period probably implies that chromosome reproduction is prevented or delayed by X-raying. If reproduction has already occurred in a great number of resting cells, as is the case in pollen-grains, prophase can start arbitrarily, the evidence of a mitose-free period thus being obscured.

The secondary effect is seen in cells starting prophase after the time of X-raying. Fragmentation and translocation phenomena occur, giving rise to acentric and dicentric structures. As regards the fragments, three types are found: unequal and identical chromosome frag-

ments (the chromatid pieces of different or equal size) and chromatid fragments. There is a definite regularity with respect to their origin. Chromatid fragments arise abundantly after X-raying prophases but also after the irradiation of resting nuclei in tissues having no mitose-free period (pollen grains). In tissues with a pronounced mitose-free period, as in dividing roots, resting nuclei produce chromosome fragments almost exclusively. By X-raying resting seeds the fragment type, seen in the germination stages immediately following, is most frequently of the double (chromosome) appearance. Now and then some fragments apparently single or unequally double are formed. In pollen grains different fragment types arise before and after a definite time. In an intermediate period both types of fragments occur, even in the same nucleus.

The most carefully studied is *Tradescantia* pollen (RILEY, MATHER, SAX). According to all investigations exclusively chromatid fragments are found when examining pollen fixed 5 hours after irradiation. RILEY and MATHER obtained no chromosome fragments for the first 24 hours. SAX found double breaks 7 hours after the X-raying (Table 1) and 24 hours (Table 3). For a short time both fragment types occur together [RILEY: 48—72 hours, MATHER: 36—72 hours, SAX 7—72 hours (Table 1) and 24—72 hours (Table 3)]. SAX has attributed the origin of chromosome fragments to a damage of two chromatids at identical level. Presumably this explanation is not correct. As mentioned above, MATHER did not find any chromosome fragments until 36 hours and RILEY not until 48 hours. Nor was SAX himself able to detect any fragments of this type during the first 7 hours. Thus, in the beginning prophase equal chromosome fragments do not arise and therefore breaks of both chromatids at identical loci *cannot* appear at this stage.

Physiological reproduction occurs at the time of highest nuclear sensitivity (MARSHAK, 1935, 1937; GUSTAFSSON, 1936, 1937; SAX, 1938). Table 3 in SAX's paper shows the following disturbance rates: 1—4 hours — 0 % breaks, 4—7 hours — 1 %, 24 hours — 11 %, 48 hours — 7 %, 72 hours — 4 %, 94 hours — 2 %. Most probably the reproduction itself is the ultimate cause of this change in stability. MATHER discovered (1937, p. 100) that pollen grains fixed 36 hours after the treatment contain both types of fragments *but usually in different nuclei.* This important statement has been disregarded by SAX. Now chromosome fragments do not appear until 24 hours after the treatment (Table 3), at the time of highest sensitivity, and chromatid fragments are formed up to the moment when the disturbance frequency is normal

again, that is, during the whole period of nuclear susceptibility. As most of the nuclei in this middle period contain either chromosome or chromatid fragments and only a few both types, this must imply that these last-mentioned nuclei were at the time of treatment at the precise moment of reproduction. If the division and reproduction rates are slowed down (as in SAX's experiments), more nuclei will be hit by X-rays at the time of reproduction and so more nuclei will show both types of fragments and the period of great susceptibility is increased. Thus we may conclude that *at the very moment of reproduction* chromosomes behave at the same time as single and double structures.

Eremurus and *Allium* pollen differ in some respects from the *Tradescantia* pollen. In the genera mentioned the physiological reproduction stage takes place at 78 and 214 hours after the treatment (MATHER), i. e. in the middle and in the beginning of the resting stage. Recent X-ray work has thus shown that the reproduction may occur at any stage of the resting nucleus, the sequence being 1) *Allium*, 2) *Eremurus*, 3) *Tradescantia* pollen and 4) germinating seeds and growing roots.

The regularity of the time occurrence of fragment types was emphasized above. No doubt, GATES's objections (1937 and 1938) to the value of X-ray work for deciding problems of chromosome reproduction are premature. However, it must be pointed out here that chromatid and unequal chromosome fragments can be produced at will after X-raying nuclei containing single chromosomes. One of the present writers has found that these two types of fragments arise abundantly after intense desiccation of resting seeds above 96 % H_2SO_4 in exsiccator and subsequent X-raying (GUSTAFSSON, unpublished). If this change is caused by dehydration phenomena or by a shift of pH in an acid direction due to the evaporation of H_2SO_4, has not been determined so far. Chromosomes may be thought to consist of long chain-molecules of polypeptids with associated H_2O-dipoles around the hydrophilous groups (OH etc.) (WRINCH, 1936; CASPERSSON, 1936; FREY-WYSSLING, 1938). The intense and rapid desiccation will cause a disappearance of H_2O-molecules around the polypeptid chains and shrinking phenomena in the intermicellar structure. In this way a »false splitting» of the chromosomes may be imitated (cf. the two- and four-thread hypothesis of chromosome structure). As the origin of chromatid fragments is correlated with a very high frequency of disturbances (almost 90—100 %), the irregularity may be due also to an imperfect reproduction after the X-raying, as postulated in earlier

cases (GUSTAFSSON, 1937, p. 294; cf. MATHER, 1937, p. 101 and NEBEL, 1937, p. 367). Problems concerning the reversibility or irreversibility of the above-mentioned process will be elucidated in further studies.

X-RAY FRAGMENTS IN SPINACIA, ZEA AND HORDEUM.

Since the seeds are X-rayed in the resting stage, all nuclei and chromosomes are of the resting type. Therefore the types of fragments necessarily depict the structure of the chromosomes in the resting stage. As shown in Part I of this paper the double chromosome reproduction in *Spinacia* occurs before the visible prophase, the determination of the actual reproduction time still being somewhat uncertain. BERGER (1938) and GEITLER (1938) have been able to prove that in their cases the rhythmical growth and the repeated chromosome division occur during the resting stage, so the conclusion may be drawn that this is also true of the periblem cells in *Spinacia*. As, however, the reproduction in the last-mentioned genus happens only twice, this must be caused by a distinct physiological mechanism, forcing the nucleus to start prophase immediately after the second reproduction or preventing a third.

Three different possibilities exist regarding the time of reproduction and the type of fragmentation in *Spinacia*. 1) Both reproductions have happened before the X-raying, the pairs of chromosome threads having already been formed. 2) One reproduction has occurred before the X-raying and the second takes place after the irradiation. 3) Both reproductions coincide with respect to time, occurring after the X-raying.

In Case 1 we can expect the following types of fragments: a) The four threads behave as distinct units. 1, 2, 3, 4 chromatid fragments are formed. There is but little chance of all the four threads releasing fragments at identical loci. b) Unequal chromosome fragments arise, consisting of fragments of different chromatid length. Different kinds are possible. c) Equal chromosome fragments occur. Either one of the two chromosomes releases an equal fragment, both of them release double fragments of different size or, finally, all the chromatids are damaged at one level, giving rise to a pair of equal and double fragments.

In Case 2 the possibilities are not so numerous. The X-rays may damage one or both of the chromatids at different or identical loci, the latter type of breaks being rarer. The second reproduction occurring, equal chromosome fragments and unequal or equal pairs of fragments arise.

In Case 3, most frequently only one type will arise. The thread, being single at the time of X-raying, will release a single fragment, which changes to the double appearance after the first reproduction. After the second reproduction *pairs of double fragments* will arise, provided of course that reproduction is a property of the »chromosome substance» and not dependent on the occurrence of centromeres.

If the chromatid, as supposed by many cytologists (SHARP, NEBEL, GATES, HUSKINS, KUWADA and others), contains two or more chromonemata instead of only one, the X-ray result should be much more complicated even in the third case where both reproductions occur after the X-raying at the onset of prophase. But as recent X-ray work has undoubtedly shown the *physiological* incorrectness of these ideas for normal mitosis, they will not be taken into account here.

These X-ray experiments have been carried out with dry resting seeds of *Spinacia*, commercial variety »Hertha», presenting a high degree of double reproduction, and for the sake of comparison with the corn-variety »Manalta» and the *Hordeum*-variety »Golden Barley» (Swedish Plant Breeding Institute at Svalöf). For genetical purposes the last-mentioned variety was treated with 5000 and 10000 r-units at the Radiological Institute of Lund University, and the *Spinacia* and *Zea* varieties at the Geological Institute. *Spinacia* was given 100 kw and 2,5 m. a. unscreened radiation at a distance of 30 cm. for 2 $^1/_2$, 3, 3 $^1/_2$ and 4 hours and *Zea* for 2 $^1/_2$ hours. In the last two genera the exact amount of r-units was not calculated. Roots just starting germination were fixed in the chromic acid-fixative used at the Svalöf Chromosome Laboratory. Longitudinal sections of all genera were prepared, in the case of *Spinacia* also numerous transverse sections. Ana- and telophases of *Zea* and *Hordeum* show immediately the type of fragment occurring. In *Spinacia* it is impossible to determine for certain from the anaphase and telophase appearance whether the preceding metaphase plates belonged to the double or the single type. Besides, the periblem cells usually contain much food material, which renders the examination of longitudinal sections fairly difficult. Therefore it was decided to study for the periblem cells exclusively metaphase plates of sections cut transversely. Fragments of the material treated for 4 and 3 $^1/_2$ hours were frequent and thus it was possible to choose for examination and drawings only very good divisions.

As has been shown previously (GUSTAFSSON, 1936, 1937) the fragments present in all tissues of barley-seeds are of the chromosome type.

This is the case in dermatogen, plerom, chalyptrogen, as well as in periblem. In this fairly superficial study no exceptions were found.

In corn identical evidence was obtained. All fragments seen, from plerom, periblem etc., are of the equal chromosome type. As no seed-

Figs. 1—3. Types of fragments in germinating seeds of corn. — × 3800.

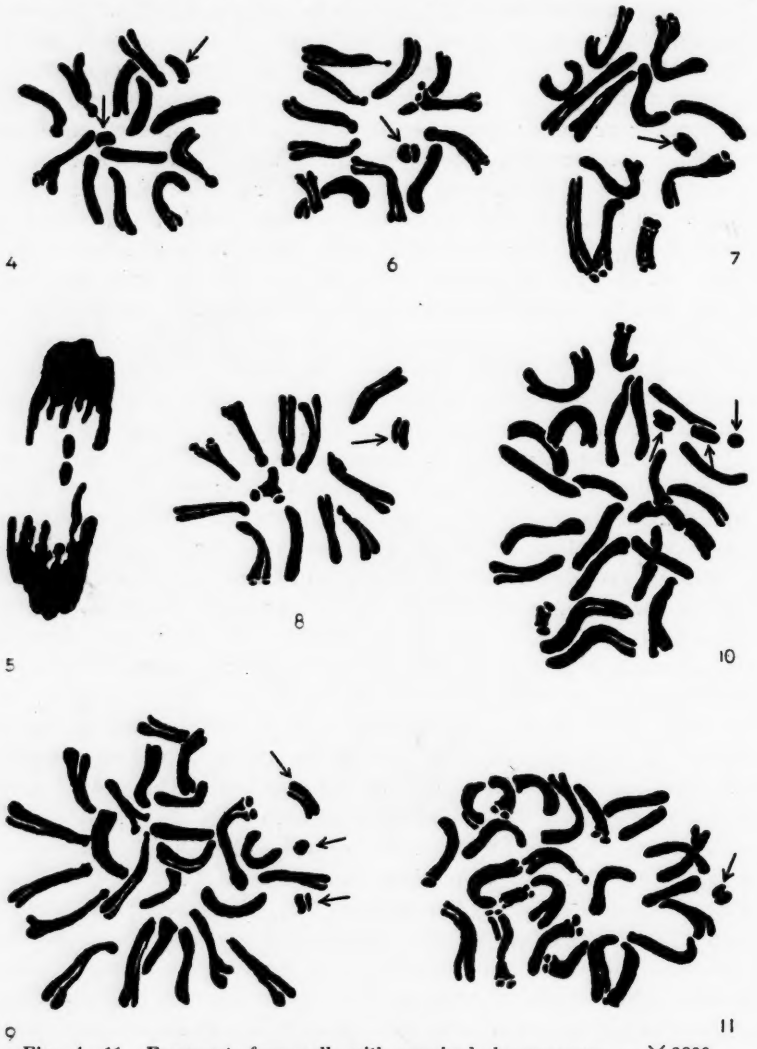
material of maize has been examined formerly with regard to this problem, some drawings are given from longitudinal sections, clearly indicating the type found (Figs. 1—3).

In *Spinacia* matters are quite different. The plerom, dermatogen and the chalyptrogen regions — all ordinarily diploid — behave as

in *Hordeum* and *Zea*. The diploid cells of the periblem act in exactly the same manner. Only equal chromosome fragments are obtained. There is a remarkable contrast between the polyploid cells with pairing (II-cells) and the diploid cells. In the former case only *pairs of double fragments* are formed, which lie a little separated from each other on the margins of the plate. In all clear II-cells with fragments we found two smaller or larger bodies, each of them having the chromosome breadth and in most cases being plainly double, the chromatids more or less sliding apart. In some ordinary prophase and metaphase plates of *Spinacia* (either in I- or in II-cells) the chromatids are attracted so strongly to each other that the double chromosome structure is not plain. So it is not surprising, especially in small and spherical fragments, that the double structure is often demonstrated solely by their breadth and mutual arrangement. Far more commonly, however, their internal construction is obvious. The fragments of II-plates differ always from those of the diploid plates in such a manner that the two chromosome fragments lie separated. In ordinary mitosis of any material the chromatids of chromosome fragments are fairly close to each other (within the same calymma?), it is only in the case of small fragments lying in the anaphase spindle that the two chromatids may repel each other and orientate themselves in the length axis similar to dividing univalents (with centromeres) at first meiotic division. In spite of the spatial separation the two chromosome fragments arrange themselves as *pairs of fragments,* usually with the mutual distance characteristic of pairs of chromosomes. No unequal pairs of fragments were found in the material examined.

Such pairs of double fragments arise in cells containing 12_{II} as well as in cells with 24_{II}. Numerous metaphase plates were studied belonging to the tetraploid and octoploid status. The cause of the double reproduction must be identical in both cases, the 12_{II}-cells originating from 12_{I}-cells, the 24_{II}-cells — on the other hand — from 24_{I}-cells. If the 24_{II}-cells had arisen directly from 12_{I}-cells, one reproduction, causing primarily the origin of 12_{II}, then another reproduction giving 12_{IV}, further the pair-wise repulsion of centromeres and chromosomes and, finally, the ordinary prophase reproduction giving the chromosomes the chromatid structure [$12_{I} \rightarrow 12_{II} \rightarrow 12_{IV}$ ($= 24_{II}$) + the normal reproduction] would have taken place, and so we should obtain four-groups of double fragments. This explanation, highly improbable in itself, can now be disregarded. We must conclude that already the resting seeds of *Spinacia* contain polyploid periblem cells.

In Part I of this paper it was shown that the increased chromosome numbers are not due to nuclear fusions or pseudo-amitotic phenomena,



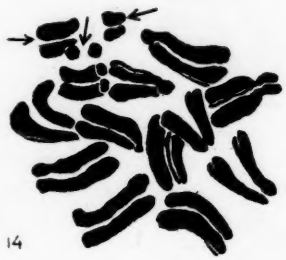
Figs. 4—11. Fragments from cells with unpaired chromosomes. — × 3800.

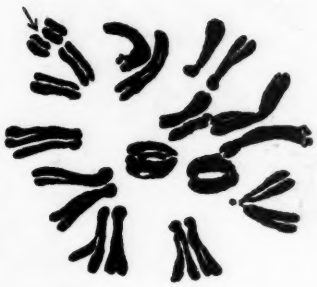
as has been continually suggested. Consequently cells with 12_{II} and 24_{I} have a different origin. 12_{II} cells go through their first mitosis after the

increased number has arisen. 24_I-cells, however, have passed through one more mitotic cycle. The few gradations occurring between 12_{II}- and 24_I-cells (showing more or less pairing) are due to an incomplete annihilation of the paired status in 24_I-cells during the preceding telo-

12

13


14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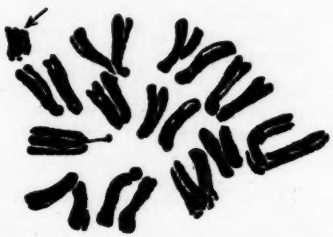
16

Figs. 12—16. Fragments from cells with 12_{II}. — × 3800.

phase and resting stage. This is proved by the different prophase behaviour. Equally convincing are also the X-ray results.

24_I-cells behave identically like diploid cells, even from the polyploid region of the periblem. A few 48_I-cells have also been found containing fragments. In all cases exclusively chromosome fragments of

the ordinary appearance were seen, *never pairs of double fragments*. Thus metaphases with single chromosomes (consisting of two chromatids) from any part of the *Spinacia*-root are physiologically alike, the degree of polyploidy causing no difference.

A brief explanation of the figures will be given in addition to the general discussion above.

Figs. 17—19. Fragments from cells with 24_{II}. — × 3800.

Figs. 1—3 are taken from ana- and telophases of *Zea mays*. In Fig. 1 one equal medium-sized chromosome fragment is seen, in Fig. 2 three such fragments (possibly four) and in Fig. 3 four double fragments occur. The long slender connections between the daughter plates are due to the stretching of dicentric configurations, which are very common. Fig. 4 is a 12_I-metaphase from the plerom of *Spinacia* showing

two chromosome fragments. Their double nature is proved by their breadth but was not quite evident under the microscope. In Fig. 5 there is a double fragment orientated in the length axis of the spindle (in our material fragments under a certain size behave in this manner; cf. CARLSSON, 1938), and the two chromatid pieces have already separated.

Figs. 6—19 are from periblem divisions of *Spinacia,* Figs. 6—8 of diploid cells. All of them show 12_I and one fragment, of different size in the three plates. Figs. 9—11 show 24_I and 3, 3, 2 chromosome fragments respectively, most of them being clearly double but never associated in pairs. (In Fig. 11 only one fragment is indicated by arrow.)

Figs. 12—16 illustrate the type of fragments in 12_{II}-cells. Fig. 12 shows 12_{II} and three pairs of fragments, all plainly double, Fig. 13 three pairs of fragments, Fig. 14 three pairs of fragments, Figs. 15 and 16 in each plate one pair of large equal-sized fragments.

Figs. 17—19 depict metaphases of 24_{II}-cells. In Fig. 17 we find $23—24_{II}$ and two or three pairs of fragments, the members of which are plainly double. Fig. 18 shows $22_{II} + 2_I$ (two chromosomes somewhat separated) and two pairs of fragments. The number 23_{II} is probably explained by the occurrence of tetracentric chromosomes (pairs of dicentric chromatids). In Fig. 19, finally, we see 23_{II} and four pairs of fragments or, possibly, 24_{II} and three pairs of fragments.

CONCLUSIONS AND DISCUSSION.

The following is a summary of the facts obtained.

In the periblem-cells of *Spinacia*-roots from germinating seeds polyploidy is frequent. This case of polyploidy is not caused by pseudoamitosis or fusion phenomena but by repeated reproductions, always to a number of two. Polyploid metaphases of two types occur, either with more or less irregular distribution of the homologous chromosomes or with a definite arrangement of chromosomes in pairs. Cells with 12_{II}, 24_{II} are frequent but 48_{II} have also been found. A secondary somatic pairing of the *Drosophila*-type cannot explain the regular distribution. That is completely proved by the prophase behaviour which has not been taken into account by earlier investigators. Pairs of chromosomes occur already here, they cannot therefore be due to a chance attraction at prophase or full metaphase. The two chromosomes of a pair are not independent but coiled around each other, in a manner similar to the relational coiling of ordinary mitosis. Thus the two chromo-

somes correspond here to chromatids. The double structure of the paired chromosomes is obvious in favourable nuclei. A pair of chromosomes consists of two independent chromosomes and four chromatids.

Polyploid cells without this conspicuous pairing originally arose in the same way but passed through telophase and resting stage after the paired metaphase-stage and changed the paired distribution to a more or less unpaired arrangement.

In germinating roots of full-grown seeds the periblem differentiation into a mixed tissue of diploid and polyploid cells starts at a certain distance from the root-tip. The order of origin is $12_I \rightarrow 24_I \rightarrow 12_{II} \rightarrow 24_{II} \rightarrow 48_I \rightarrow 48_{II}$. As cells with 24_I are found earlier than cells with 12_{II}, this implies that the double reproduction force in germinating seeds cannot act until a certain distance from the tip but has acted closer to the chalyptra (in a relative and absolute sense) earlier during the embryo-development.

There is conspicuous parallelism between chromosome number and size of the cells. 12_{II} and 24_I are larger than 12_I; 24_{II} and 48_I larger than 12_{II} and 24_I. 48_{II}, finally, are larger than both 24_{II} and 48_I. The polyploidy present in *Spinacia* is essentially identical with the type arising after auxin-treatment (LEVAN, 1939) in such tissues where cell elongation and cell growth are possible. Except for the difference with regard to the time of centromere division both have all characteristics in common. In LEVAN's case centromere division was delayed. In *Spinacia* each chromosome of a pair obtains its own centromere apparently at the beginning of prophase. Presumably this difference in the behaviour of centromeres is due to difference in material, since auxin-treated cortical cells of *Spinacia* show exactly the same type of chromosome pairs as periblem cells (Part III of this paper). LEVAN came to the conclusion that the effect of auxin is primarily a cell-stretching phenomenon, this explanation being in line with the previously known effect of auxin and with the results obtained in *Spinacia* (cf. below). Cells with 12_{II} are smaller than those with 24_I and cells with 24_{II} than cells with 48_I. This argues in favour of the view that polyploid metaphases with single chromosomes have actually passed through one more mitotic cycle.

The double reproduction must take place in a stage previous to the earliest prophase where chromosomes can be examined. From the known behaviour of polyploid nuclei in Diptera, *Culex* and *Gerris* it

may be concluded that the reproduction takes place somewhere during the resting stage, a conclusion in agreement with the X-ray results.

Ordinary diploid cells of barley, corn and *Spinacia* give almost exclusively chromosome fragments. II-cells, either of the 12_{II}- or the 24_{II}-type, produce pairs of chromosome fragments. In polyploid I-cells (24_I and 48_I), however, only chromosome fragments of the ordinary type (double and equal) are formed. If the X-ray methods are valid, this indicates that the resting chromosomes are single (at any rate they are *physiologically* single). The double reproduction is therefore restricted in time to the transition of resting stage to prophase. Chromosomes in II-cells are more contracted than those of I-cells. This probably implies that from the very onset of prophase to full metaphase a longer time is required. This contraction process is more pronounced in the case of 12_{II}, 24_{II}, 48_{II} than in the case of 24_I, 48_I. Thus the double reproduction itself should in some way or another be responsible for the higher degree of contraction.

From the type of fragments obtained in II-cells we must conclude that the reproduction properties of the chromosomes are independent of any morphological structures (centromeres, satellites, secondary constrictions) but also independent of the nucleic acids accumulated during the course of mitosis. Fragments lacking a centromere reproduce in the same manner as the remaining chromosome bodies. If the nuclei pass through two internal reproductions, they reproduce twice. Since CASPERSSON has shown that the augmentation of nucleic acids in mitosis does not start until the beginning of prophase, this synthesis is probably not connected immediately with the reproduction. The view expressed by FREY-WYSSLING (1938) that nucleic acids primarily have a »gene-inhibiting action» (by means of salt-formation during the kinetic phase) agrees with these results.

We also find a uniform nuclear control of the reproduction. All chromosomes of a nucleus reproduce simultaneously (or practically so). That is true of normal mitosis, *Spinacia*-mitosis and the origin of polyploid nuclei in *Culex* and *Gerris*. BERGER found no exceptions to the 2^n-rule, this in contrast to earlier investigations. By counting the number of X-chromosomes of the male in *Gerris*, GEITLER discovered so far only the numbers 1, 2, 4, 8, 16, 32, . . . In *Spinacia* hundreds of metaphases have been examined, showing no 3x, 5x, 6x, 7x or $2x + 1$, $2x + 2$ numbers etc. but exclusively 2x, 4x, 8x and 16x ($x \cdot 2^1$, $x \cdot 2^2$, $x \cdot 2^3$, $x \cdot 2^4$). This general control of the nuclear reproduction must be

due to distinct chemical properties of the resting chromosome material and to definite changes at the time of reproduction.

The mechanism of the double reproduction will be discussed more in detail in future papers on its physiology and chemistry. Here only a short outline of its theory will be drawn.

Auxin has no clear karyological effect on purely meristemic tissue. Where cell elongation and cell growth can occur for physiological and mechanical reasons, chromosomes are forced 1) to reproduce and the nucleus to divide after a minimum growth of the cell (the nucleus-plasm relation; HERTWIG). If, however, growth is too strong or too rapid, 2) chromosomes will reproduce twice before the prophase starting force can act. Similarly in *Spinacia.* In resting seeds many cells of the periblem, where the occurrence of intercellulars and the structure of the tissue make a considerable degree of growth possible, have acquired a size that does not correspond to the chromosome number. Still the diploid number may not be increased at the time of germination. If, however, the cell-size has grown above a certain threshold value, reproduction must continue until cell-size and nucleus size (nuclear surface? ABELE, 1936) are in harmony. As nucleus size is proportional to the chromosome number, an increase in nucleus size can be brought about by internal reproductions. Consequently we may conclude — in order to obtain a correct understanding of the cell-nucleus relationship — that special substances are accumulated in the cytoplasm, acting on or interfering with the reproduction inside the nucleus. If their concentration, which ought to be proportional to the amount of cytoplasm, due to an inhibited cell growth is too low, chromosome reproduction and cell division will be prevented; if it is in harmony with the amount required for an ordinary mitosis, reproduction will be normal; if it is much too high — as is the case supposedly in *Spinacia, Culex* and so on — reproduction must be repeated. This accumulation of substances (reproduction forces) should therefore be proportional to the amount of cytoplasm and in this way to a certain degree correlated with cell growth and cell elongation.

Previously one of the writers has shown that growth and vacuolisation phenomena change the meiotic disposition in mitotic direction (GUSTAFSSON, 1939). This state of things seems to be a general rule. In accordance with the above argumentation reproduction substances are accumulated during this growth process of cytoplasm and cell-wall. They counteract the meiotic tendency and cause a chromosome reproduction already in the pre-meiotic resting stage. Since vacuolisa-

tion and cell growth can be very marked in the case of apospory (*Alchemilla* and *Artemisia*) without any increase of the chromosome number, the accumulation of special reproduction substances should be due to the amount of *active* cytoplasm as postulated above.

Internal reproductions occur most frequently in tissues with secretory functions. That is true also of *Spinacia*. The polyploid periblem cells contain a large amount of stored food material. Whether this storage process is a primary cause of the increased chromosome number or a secondary consequence has not been determined so far.

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STUDIES ON THE PROPERTIES AND THE WAYS OF PRODUCTION OF RYE-WHEAT AMPHIDIPLOIDS

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AMONG experimental allopolyploids the *Triticale* types, containing the genomes of wheat and rye, are of special interest not only from a theoretical but also from a practical point of view. Since wheat and rye are both cereals of a high economical value their synthetic products, the *Triticale* strains, might, indeed, be expected to have remarkable properties. As is well known, however, the observations hitherto made on *Triticale* strains gave rather negative results as regards their practical value. In the first place this is due to the low yield, the poor seed setting being correlated with more or less profound meiotic irregularities.

The present writer has been engaged in *Triticale* experiments since 1934 and some of the results of this work have already been published (MÜNTZING, 1935 a, 1936 a, 1938 a, 1938 b). Though the results are still in many respects fragmentary, I now find it appropriate to give a summarizing report of the observations so far made concerning the properties of *Triticale* strains and the different ways of their production.

I. CONSTANT TRITICALE STRAINS AND THEIR PROPERTIES.

1. MATERIAL.

Six constant (or relatively constant) *Triticale* strains were studied by the present writer. These strains are the following: *Triticale* RIMPAU, a bearded form of RIMPAU kindly sent to me by Prof. E. VON TSCHERMAK, »*Triticale* Svalöf» (the origin of which was described in my paper of 1936 a), finally three *Triticale* strains obtained from U. S. A. Two of these were kindly placed at my disposal by Dr. J. W. TAYLOR, one being of Russian origin, the other representing the wheat—rye amphidiploid described by TAYLOR and QUISENBERRY (1935). The third *Triticale* type from U. S. A. was kindly given to me by Dr. G. A. WIEBE, Davis, California. Originally this strain was thought to be the one

produced by TAYLOR and QUISENBERRY and was thus in my 1936 a paper called »*Triticale* TAYLOR». It was subsequently found, however, that the WIEBE strain and the TAYLOR and QUISENBERRY strain were quite different. The WIEBE strain, on the other hand, is rather similar to the Russian *Triticale* type obtained from Dr. TAYLOR and is probably also of Russian origin. However, as far as our evidence goes the two strains in question are really different in some respects (cf. below p. 395).

In order to simplify the denomination of the six constant strains they will simply be referred to as *A*, *B*, *C*, *D*, *E* and *F*. The key to these denominations is the following:

A = *Triticale* RIMPAU
B = » » bearded
C = The WIEBE strain (probably of Russian origin)
D = Russian *Triticale* strain, obtained from J. W. TAYLOR
E = *Triticale* TAYLOR and QUISENBERRY
F = *Triticale* Svalöf

2. FERTILITY.

A. POLLEN FERTILITY.

Pollen fertility in the six *Triticale* strains was found to be less good than in *Triticum vulgare*. In the years 1934—1937 a total of 22 pollen samples of various *vulgare* strains gave an average of 96,6 per cent. good pollen. During the same period the corresponding values of the *Triticale* strains tested were found to be the following: strain *A* = 92,5 (10 samples), strain *B* = 77,5 (5 samples), strain *C* = 89,2 (9 samples), strain *E* = 72,5 (3 samples), strain *D* = 80,0 (4 samples) and strain *F* = 70,8 (15 samples). Besides these fragmentary values more precise information as to the pollen fertility of *A* and *C* was gathered in 1938. (Table 6, p. 406). In that year 35 plants of *C* gave the average value 78,1, the corresponding figure for 42 *A* plants being 74,8. Since the distribution in *A* (and the corresponding F_1 and F_2) is bimodal, the standard errors were not calculated. Nevertheless the partial sterility of the two strains is evident, this being in accordance with the relatively poor pollen quality previously observed in these strains and others.

This summer (1939) pollen samples were taken from strains *A*, *B* and *F*, these strains being grown in small plots side by side. The samples are therefore strictly comparable. The percentage of good pollen was found to vary as follows:

Strain	Percentage of good pollen										n	M ± m
	10	20	30	40	50	60	70	80	90	100		
A						2	9	12	6		29	82,6 ± 1,6
B						2	14	8	3		27	79,4 ± 1,6
F	1	1	2	1	4	11	5	2	1		28	61,8 ± 3,4

Pollen fertility in strain *F* is evidently less good than in *A* and *B*, the differences *A* — *F* and *B* — *F* being quite clear and significant (D/m = 5,5 and 4,6 respectively).

B. SEED SETTING.

In 1934 a preliminary estimation of seed setting in strain *C* was undertaken. Ten open-pollinated ears, taken at random, were found to contain from 18 to 33 kernels per ear, the average being 23,3. In nine isolated ears the number of seeds ranged from 17 to 32 with an average value of 24,9. Thus, in strain *C* seed setting after isolation seemed to be just as good as after open pollination, and the conclusion was drawn that this *Triticale* strain was a good self-fertilizer. Since not only *Triticale C* but also the other *Triticale* strains studied are morphologically constant or almost constant, it is evident that in *Triticale* self-fertilization is the rule, just as in *Triticum vulgare*. At any rate, the *Triticale* strains are in this respect much more similar to wheat than to rye, which is typically self-sterile. In comparison to wheat, however, seed setting in *Triticale C* was found to be very low. In ten ears of an ordinary wheat variety, studied on the same occasion, the number of seeds per ear varied between 39 and 78 with a mean of 55,2. This value is more than twice as high as in *Triticale C*.

In 1937 and 1938 some further data on seed setting in the *Triticale* strains were acquired. The results of the former year are given in Table 4, p. 403. As is evident from the table, the number of seeds per spikelet is low in both the *Triticale* strains compared, the values of *A* and *C* being 1,40 ± 0,05 and 1,50 ± 0,05 respectively. Isolation of the ears gave no significant decrease of the seed setting, thus supporting the previous results in *Triticale C*.

In 1938 the *Triticale* strains were grown in two different fields, I and II. At the harvest the strain *A* material from field I was unfortunately spoilt, due to the fact that the ears unexpectedly proved to be rather brittle and thus were to a great extent broken. Material of *Triticale C* from the same field could be used and gave an average value of 1,32 ± 0,03 seeds per spikelet (Table 5, p. 405). From field II, where

the plants had been more widely spaced than in field I, material of *C* as well as *A* were available. These plants gave the values $1{,}55 \pm 0{,}05$ and $1{,}49 \pm 0{,}07$ respectively, thus again indicating a better seed setting in *C* than in *A*.

Also in the morphological quality of the seeds there is a marked difference between *A* and *C*. In 1938 the degree of shrivelling of the seeds was estimated, using a scale from 1 to 10, the higher values representing the better qualities. In material from field II the average values in this respect were found to be $4{,}80 \pm 0{,}22$ in *C* and $4{,}01 \pm 0{,}23$ in *A*. The chance of this difference being significant is 74 to 1 ($D/m = 2{,}47$).

In 1938 preliminary data on the seed setting in some other *Triticale* strains were also obtained. In strain *B* the average value was found to be $1{,}43 \pm 0{,}12$ and in strain *D* $1{,}17 \pm 0{,}07$, these values being comparable to the average values of *C* and *A* just mentioned ($1{,}55 \pm 0{,}05$ and $1{,}49 \pm 0{,}07$ respectively). The difference between the average values of *C* and *D* is evidently significant ($D = 0{,}38 \pm 0{,}09$). — Thus, taking all the data into consideration, it is evident that *the Triticale biotypes tested are all partially sterile, but the degree of this sterility is different in different strains.*

3. MEIOSIS.

So far meiosis has only been studied in two of the constant strains, viz. *A* and *C*. The observations have for the most part been limited to counts of the number of univalents. As is already well known, the presence of a variable number of unpaired chromosomes at I—M is a typical feature of *Triticale* strains. This was also found to be true of the material studied by the present writer (Figs. 3—4). The results are summarized in Table 1.

As is evident from the table, different fixations and even different preparations of the same fixation may give rather different values. Similar experiences were made by VON BERG and OEHLER (1938). On an average, however, *it is obvious that the frequency of univalents is much higher in A than in C.* The total average of *A* is not less than 6,10, and in this strain not a single entirely regular first metaphase group without univalents was observed. A corresponding result with the same strain (Rimpau) was obtained by VON BERG and OEHLER (1938), who found an average number of $5{,}75 \pm 0{,}53$ univalents. In our strain *C*, on the contrary, the maximum class corresponds to 0 univalents, and the total average frequency is as low as 1,88.

TABLE 1. *Number of univalents at I—M in two Triticale strains and their hybrids.*

Strain	Fixation and slide number	Number of univalents at I—M																			n	M
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
C	219. P. 1	50	1	26	3	7	1	3	—	—	1	2	—	1							95	1,62
»	219. P. 2	66	5	41	5	18	2	4	2	2	—	1	—	—	—	—	2	—	—	2	150	2,09
»	220. P. 1	22	1	20	—	7	1	2	—	—	1										54	1,76
C	Total	138	7	87	8	32	4	9	2	2	2	3	—	1	—	—	2	—	—	2	299	1,88
$C \times A$, F_1	582. P. 1			1	1	2	3	5	3	6	2	5	—	1	1	—	—	—	—	1	31	7,68
»	578. P. 1				1	1	1	3	6	8	3	3	1	4	3	1					35	8,71
»	578. P. 2					1	—	1	—	6	—	3	1	3	1	1	1				18	9,83
$C \times A$, F_1	Total			1	2	4	4	9	9	20	5	11	2	8	5	2	1	—	—	1	84	$8,57 \pm 0,33$
A	481. P. 1				1	—	2	5	15	32	14	8	1	4							82	8,16
»	482. P. 1							3	10	13	12	11	4	3	1						57	8,82
»	482. P. 2			3	18	11	35	6	17	5	7	3	1	—	1						107	4,52
»	584. P. 1			6	15	7	20	7	9	1	2	1									68	3,79
A	Total			9	33	19	55	15	34	31	54	30	20	5	8	1					314	$6,10 \pm 0,16$

The occurrence of univalents in these strains may be interpreted as non-conjunction in a variable number of pairs of homologous chromosomes. This is evident from the fact that the classes with an even number of univalents represent higher frequencies than those with odd numbers. In *C* this is true of the classes with 2, 4 and 6 univalents, and in *A* this preponderance also includes the class with 8 univalents. In cells with a still higher number of chromosomes this regularity disappears, probably due to the fact that in such cells the exact number of univalents is difficult to distinguish.

Besides unpaired chromosomes bivalents were almost exclusively present at I—M in both the strains studied. In *A* a trivalent occurred in one cell, and in *C* a single association of four chromosomes was seen. Undoubtedly, however, the configurations in these strains are quite predominantly made up of a variable number of bivalents and a corresponding number of univalents. In a few pollen mother cells of *C* with quite regular metaphase groups all the 28 bivalents could be distinguished. Fig. 1 shows the chromosome complement in such a case. In this group seven bivalents are rod-shaped with one terminal chiasma, the other bivalents have two or more chiasmata.

When counting the number of univalents, it was observed that in some parts of the anthers the frequency of univalents was decidedly higher than in other parts. This strongly indicates that in this material the degree of pairing is not only a function of the chromosomal homology but is also conditioned by somatic influences.

The presence of univalents at I—M is responsible for various irregularities occurring at later stages. Thus, as already reported by VON BERG and OEHLER (1938), division of univalents is frequent at I—A, and at II—A the split univalents are distributed at random to the poles. — On an average the number of chromosomes dividing at I—A was found to be lower than the number of univalents present at I—M. This is evident from the following figures: In *C* (R. 219, P. 2) the average number of univalents at I—M was found to be 2,09 (cf. Table 1). In the same slide 32 cells at I—A gave an average of 1,18 univalents dividing. In another preparation of *C* (R. 220) the corresponding figures were 1,76 (54 cells) and 1,02 (46 cells). In two slides of strain *A* the following average values were obtained. I—M in R. 584 showed 3,79 univalents (68 cells), I—A in the same slide 3,07 univalents (14 cells). Finally, in the other slide of *A* (R. 481) the number of univalents at I—M and I—A was found to be about the same, the average values being 8,16 (82 cells) and 8,18 (12 cells) respectively.

Figs. 1—4. First metaphase in *Triticale C.* — Fig. 1, side view, separately drawn, 28_{II}; Figs. 2—4, cells showing different degrees of regularity; Fig. 2, no univalents and only bivalents visible; Fig. 3, 6 univalents; Fig. 4, extreme non-conjunction, 18 univalents. — × 3175.

The lower average number of univalents at I—A, as compared to I—M, shows that some of them are included in the interphase nuclei without division.

4. VIGOUR.

The *Triticale* strains differ from each other not only in fertility and degree of meiotic regularity but also in vigour. In 1939 the six strains were grown in small plots side by side. Plant height in this material was found to be as follows (Table 2) [1].

TABLE 2. *Plant height in six Triticale strains.*

Strain	Plant height													n	M ± m
	20	30	40	50	60	70	80	90	100	110	120	130	140 cm.		
F			1	3	7	5	16	11	14					57	76,2 ± 2,1
A									3	5	7	16	9	40	120,8 ± 1,9
B						1	2	3	5	8	18	11	2	50	110,0 ± 2,2
E						1	2	7	15	10	9	1		45	98,8 ± 1,9
C							2	1	6	14	19	8		50	109,2 ± 1,7
D	1	—	—	2	—	6		9	18	8	2			46	89,8 ± 2,4

Most of the differences between the average values are evidently significant. The most pronounced contrast is that between strains *A* and *F*, the average height of *A* (120,8 ± 1,9) being not less than 59 per cent. higher than that of *F* (76,2 ± 2,1). — It is interesting to note that *Triticale* RIMPAU (strain *A*) and bearded RIMPAU (strain *B*), which are most probably closely related (cf. TSCHERMAK-SEYSENEGG, 1937), differ significantly in height, *A* being the taller strain. According to Table 2 the difference between their average values amounts to 10,8 ± 2,90 cm. (D/m = 3,72). In the preceding year height measurements were also undertaken, the average values of *A* and *B* in that year being 147,0 ± 1,5 and 122,1 ± 1,8 cm. respectively. Thus, it is clear that the differences between *A* and *B* are not only limited to the presence or absence of awns.

Another noteworthy difference is that between *C* and *D*. In 1939 the average values of these strains were found to be 109,2 ± 1,7 and 89,8 ± 2,4 cm. respectively (Table 2). In 1938 the corresponding average values were 136,5 ± 1,5 and 109,7 ± 3,1 cm. Thus, in both years strain

[1] In the table the strains are given in the same order as they were grown in the field.

D was found to be less vigorous than *C*. Further, since *D* is probably less fertile and less winter hardy than *C*, it seems safe to conclude that the WIEBE strain (*C*) is different from the Russian *Triticale* type (*D*) obtained from Dr. TAYLOR. Morphologically, however, the two strains are very much alike and may be assumed to have a similar origin. An F_2 generation grown in this year from hybrids between *C* and *D* does not show any obvious segregation in morphological characters.

Strains *A* and *C*, which are the parents in the *Triticale* cross most closely studied (cf. below pp. 405—409), are clearly different in vigour. Strain *A* is tall and erect with a thick straw, strain *C*, on the contrary, being much more slender and with a poor straw-stiffness. This is reflected by a significant height difference. In Table 2 the average values of these strains are $120{,}8 \pm 1{,}9$ and $109{,}2 \pm 1{,}7$ cm. On another field measurements, also performed this summer, gave the values $131{,}9 \pm 0{,}8$ for *A* and $115{,}7 \pm 0{,}6$ for *C*. The same strains were also compared in the previous year, the average height values in that year being $147{,}0 \pm 1{,}5$ and $136{,}5 \pm 1{,}5$ respectively (Table 5).

5. PHYSIOLOGICAL AND CHEMICAL PROPERTIES.

Thanks to preliminary investigations performed by Prof. Å. ÅKERMAN and his collaborators at the Plant Breeding Institute of Svalöf (Doctors LINDBERG, ANDERSSON and GRANHALL), it is now clear that the *Triticale* strains are rather interesting from a chemical and physiological point of view [1]. It is also clear that the strains differ from each other in these respects. Thus, as regards winter hardiness *Triticale C* was found to be excellent, this strain being superior in this respect to most of the Swedish commercial varieties of wheat. The hardiness of *C* was, in fact, found to be comparable to that of the very hardy native strains from Finland. *Triticale A* has also been found to have good hardiness, but seems to be somewhat less good than *C*. *Triticale F*, finally, has a rather poor hardiness.

So far the chemical properties and the baking capacity have only been tested in *Triticale C*. A detailed account of these studies will soon be published by Prof. ÅKERMAN. With his permission, however, it may be mentioned already now that the percentage of raw protein and gluten was remarkably high in strain *C*. Corresponding to this the bread volume obtained was quite favourable. In a baking test the bread

[1] The detailed results of these studies will be published in future papers by the gentlemen mentioned.

volumes of the wheat varieties »Stål», »Standard» and »Turkey» were found to be 644, 582 and 764 cm.^{3} respectively. The corresponding figure of *Triticale C* was 789, thus surpassing all the other values, including that of »Turkey». Prof. ÅKERMAN has informed me that with respect to baking capacity »Turkey» is one of the very best winter wheat varieties known.

From other investigations it is evident that the *Triticale* strains have not always the same good baking capacity as *Triticale C*. Thus VON ROSENSTIEL (1938) reports results with RIMPAU (= strain *A*), clearly demonstrating a rather poor baking capability in this strain.

In this connection it may be mentioned that on an average the *Triticale* strains are intermediate in earliness between rye and wheat. As is natural, however, considering their widely different origin, the *Triticale* strains, when compared with each other, show a considerable variation in earliness. Thus, strain *C* flowers much earlier than, for instance, *A* and *F*.

II. F_1 COMBINATIONS.

1. DIFFICULTIES OF HYBRIDIZATION.

Crosses between different constant *Triticale* strains were commenced in 1936 and were continued in the following years. Much to our surprise it was found to be far more difficult to hybridize *Triticale* strains than, for example, to cross different varieties of wheat. The partial sterility characteristic of the *Triticale* strains may of course be responsible for a large proportion of these difficulties but, apparently, this does not solve the entire problem.

In 1936 *Triticale A* and *C* were hybridized, and at the same time some control crosses ($A \times A$ and $C \times C$) were also made. The results of these crosses were the following:

Cross combination	No. of flowers crossed	Percentage of seed setting
$A \times C$	800	$0{,}63 \pm 0{,}28$
$C \times A$	1532	$4{,}11 \pm 0{,}51$
$C \times C$	69	$14{,}49 \pm 4{,}24$
$A \times A$	54	$24{,}07 \pm 3{,}52$
$P \times P$	123	$18{,}70 \pm 3{,}52$

The results are obviously rather peculiar. In the first place it should be observed that the control crosses gave a higher percentage of seed setting than the crosses between *A* and *C*. The sum of the

control crosses ($P \times P$) gave the value $18{,}70 \pm 3{,}52$, and this percentage is significantly higher than the values resulting from both of the hybrid combinations ($18{,}70 - 0{,}63 = 18{,}07 \pm 3{,}53$; $18{,}70 - 4{,}11 = 14{,}59 \pm 3{,}56$). Thus, though *A* and *B* have the same chromosome number and are to be considered as different strains of the same species, the crossing experiments indicate the presence of a barrier of incompatibility between them. However, before conclusions can be drawn on this point, it will be necessary to repeat the crosses and, especially, to perform the control crosses on a larger scale.

Another unexpected result is also evident from the hybridizations in question, *viz.* that the cross $C \times A$ is more successful than the reciprocal combination. The difference between the percentage values is significant ($4{,}11 - 0{,}63 = 3{,}48 \pm 0{,}58$). Why the *C* strain is a more successful mother in these crosses than *A* is at present simply a fact, and the cause of this difference is not known. It is true that the percentage of seed setting in *A* is probably slightly lower than in *C* (cf. Tables 3—4), but this difference seems to be too slight to explain the marked difference between the reciprocal combinations. Moreover, in the control crosses $A \times A$ gave just as good or even better results than $C \times C$.

In 1937 the crosses between *A* and *C* were repeated. On an average the percentage of seed setting in that year was much higher than in 1936, but the same difference between the reciprocal combinations was observed. In the combination $C \times A$ 146 seeds were obtained from 1031 flowers crossed, this giving the precentage value $14{,}16 \pm 1{,}09$. In the reciprocal combination 36 seeds were obtained from 1100 flowers, which corresponds to a percentage value of $3{,}27 \pm 0{,}54$. The difference between these values is $10{,}89 \pm 1{,}22$ and is evidently significant.

In the same year nine other cross combinations were made, involving the *Triticale* strains *B*, *D* and *C* besides *A* and *C*. The total result of these nine cross combinations was 328 seeds from 4808 flowers, which corresponds to 6,82 per cent. The extreme values found were 0,00 and 16,17 per cent. in the combinations $A \times E$ (270 flowers) and $C \times D$ (235 flowers) respectively. — Thus, a rather low percentage of seed setting seems to be characteristic of crosses between *Triticale* strains in general.

In six of the nine new crosses referred to, both the reciprocal cross combinations were made. In two of these cases differences in crossability between the reciprocal combinations were observed just as in the crosses between *A* and *C*. Thus, from the cross $C \times D$ 38

seeds were obtained from 235 flowers, but in the reciprocal combination only 3 seeds resulted from 141 flowers. This gives the percentage values $16{,}17 \pm 2{,}40$ and $2{,}13 \pm 1{,}21$ respectively. The difference is $14{,}04 \pm 2{,}69$ and $D/m = 5{,}22$. In the other case, $D \times B$, 31 seeds were obtained from 318 flowers, but the reciprocal combination only resulted in 4 seeds from 364 flowers. The corresponding percentage values are $9{,}75 \pm 1{,}67$ and $1{,}10 \pm 0{,}55$ respectively. The difference, $8{,}65 \pm 1{,}76$, is significant ($D/m = 4{,}91$). In the four other crosses of 1937, in which both the reciprocal combinations were made, no significant differences between the two directions occurred.

In 1938 the *Triticale* hybridizations were supplemented by crosses between *Triticale F* and the five other strains. Further, *A* and *C* were crossed with *E*. All these crosses were only made in one direction. The total values of the 1938 crosses were 98 seeds from 2586 flowers, which corresponds to an average seed setting percentage of only 3,79.

In order to find out whether different degrees of male or female fertility of the *Triticale* strains might possibly influence the result of the crosses, the total seed setting percentage of the crosses between one particular line and the other lines was calculated.

Used as *male parents* the different strains involved gave the following results in crosses with the other strains [1]:

A: $7{,}92 \pm 0{,}52$ per cent.;		*F*: $3{,}61 \pm 0{,}39$ per cent.;	
B: $7{,}79 \pm 0{,}97$ »	»	*C*: $2{,}10 \pm 0{,}31$ »	»
D: $6{,}54 \pm 0{,}72$ »	»	*E*: $2{,}04 \pm 0{,}56$ »	»

Several of these values are significantly different, but so far the causes underlying these differences are not known. As reported above *Triticale C* was found to be as fertile as or even somewhat more fertile than *Triticale A*. This is true also of the pollen quality. Since, in spite of this, *A* was significantly more successful as male parent than *C*, it is obvious that differences in pollen quality of the male parent cannot explain the differences in seed setting observed. As there is probably always a sufficient number of functional pollen grains available to secure an efficient fertilization, it would seem more probable that the degree of female fertility is in the first place responsible for the result of the crosses.

Used as *female parents* the different strains involved gave the

[1] These values are based on a total of 494 seeds, obtained from 9726 cross-pollinated flowers. Thus, the average seed setting percentage of all crosses hitherto made between our *Triticale* strains amounts to 5,08.

following seed setting percentages when pollinated with the other strains:

C: 8,00 ± 0,46; *A*: 3,06 ± 0,29
D: 4,97 ± 0,64; *B*: 2,55 ± 0,46
E: 4,91 ± 1,28;

The 6th strain, *F*, is not represented among the above values, as hitherto this strain has only been used as male parent in the crosses. — As in the case of the male parents, there are significant differences between several of the average values. *Triticale C*, for instance, has been much more successful as a mother than *A*. It is of interest, however, to note that *A* as the female parent gave less good results with *C* than with the other strains. Thus, a total of 1900 *A* flowers pollinated with *C* pollen gave a seed setting percentage of only 2,16 ± 0,34. A total of 1667 *A* flowers pollinated with the pollen of the four other *Triticale* strains involved gave a percentage of 4,08 ± 0,49. The difference between the two values is 1,92 ± 0,60 and is thus significant (D/m = 3,20).

Pending more thorough comparative investigations of the male and female fertility of the six strains used for the crosses, it is at present difficult to make an accurate and consistent interpretation of all the data so far accumulated. The following conclusions, however, may be drawn already now:

a) The *Triticale* strains are difficult to hybridize, the average seed setting of all crosses hitherto made being as low as 5,08 per cent.

b) The reciprocal combinations in some crosses give significantly different results. In part at least these differences must be caused by other factors than the degree of male and female fertility of the strains crossed.

2. HYBRID VIGOUR.

Since it was found to be rather laborious to produce F_1 hybrids between different *Triticale* types, most of the F_1 individuals obtained were wintered in hotbeds, and during that time they were divided vegetatively several times. This procedure, which resulted in many clone plants, had the disadvantage of destroying the direct comparison between parents and F_1 individuals, the parents in most cases being sown directly in the field. However, though rather meagre, the data available indicate that the F_1 plants are generally more vigorous than the parental biotypes.

In 1937 clone plants from the cross $C \times A$ as well as some material

of the parent strains were available for observation. Parents and F_1 plants had been treated in approximately the same way. Plant height was measured and found to have the following values (Table 3).

The average height of *C* and *A* was $72{,}7 \pm 2{,}2$ and $79{,}4 \pm 5{,}0$ cm. respectively. The corresponding value of the F_1 plants was as high as $94{,}7 \pm 1{,}7$ cm. If the values of both parent strains are added together into one series, the mean value of this series will be $75{,}8 \pm 2{,}6$ cm. The difference $94{,}7 - 75{,}8 = 18{,}9 \pm 3{,}4$ is evidently significant ($D/m = 5{,}6$). Thus, judging from these results, the F_1 hybrids were undoubtedly characterized by hybrid vigour, the average value of the parents being much lower.

Since these measurements were made on clone plants transplanted

TABLE 3. *Plant height in the cross Triticale A × C.*

Strain or hybrid combination	Plant height											n	M ± m
	30	40	50	60	70	80	90	100	110	120	130 cm.		
A	2	1	1	3	5	4	2	3	1	1		23	$79{,}3 \pm 5{,}0$
C		2	—	8	8	8						26	$72{,}7 \pm 2{,}2$
A + *C*	2	3	1	11	13	12	2	3	1	1		49	$75{,}8 \pm 2{,}6$
F_1			1	1	6	16	17	19	7			67	$94{,}7 \pm 1{,}7$

into the field in spring, it seemed desirable to repeat the measurement on material sown directly in the field in autumn. Such material was available in 1938, plenty of F_1 plants from both reciprocal combinations having been grown in that year in addition to the parent strains and F_2. As mentioned above, the ears of RIMPAU unfortunately turned out to be somewhat brittle and were thus broken at the harvest. This destroyed the direct comparison by measurements between F_1 and both parents in field I. However, before the harvest the F_1 plants were observed to reach about the same height as *A*, the taller parent. The average height value of F_1 was found to be $161{,}3 \pm 1{,}3$ cm., that of *C*, the weaker parent, was $146{,}8 \pm 1{,}4$ (Table 5). In another field (II), where *C* and *A* could be compared, they were found to have the average values $136{,}5 \pm 1{,}5$ and $147{,}0 \pm 1{,}5$ respectively. Assuming the same height relation in field I, the average value of strain *A* in this field would be 158,1. Thus, though accidentally spoilt on the whole, the measurements of 1938 may be said to confirm those of the preceding year. At any rate, *it is obvious that the F_1 plants of this combination surpassed the average vigour of the parents,* and they even seem to be

somewhat taller than the tallest parent. They were observed, however, to be somewhat less coarse and stiff than strain *A*, the difference between the parents in this respect being considerable.

In height and general appearance the F_1 hybrids of both reciprocal combinations were quite similar. Thus, the difference in crossability of the two directions is not correlated with morphological differences between the resulting F_1 plants.

In 1938 a number of other F_1 combinations of crosses between the different *Triticale* strains were in culture, but all these F_1 plants had been divided vegetatively, and comparable material of the parent strains was not available. In comparison with other hybrids and hybrid derivatives of various kinds, which had been treated in the same way, the vigour of these F_1 plants was rather striking. This indicates that exact measurements will probably reveal similar relations between parents and F_1 hybrids as in the case of *A* and *C*.

3. POLLEN FERTILITY.

In 1938 pollen fertility was studied in *A* and *C* and in F_1 and F_2 of the cross between these strains. The results are given in Table 6. Pollen fertility in the parents has already been discussed above (p. 388), the average values of *C* and *A* in this test being 78,1 and 74,8 respectively. The F_1 hybrids were also partially sterile, the average of 66 plants being 69,7 per cent. good pollen. The distribution of the variates in F_1 is probably bimodal as in *A* (and F_2), and therefore the standard errors have not been calculated. It is obvious, however, that the F_1 plants are more sterile, on an average, than the *C* parent, and it seems probable that they are also somewhat more sterile than *A*.

In the same year a total of 45 pollen samples were gathered from eight other kinds of F_1 hybrids from crosses between the *Triticale* strains. The percentage of good pollen in these samples varied between 30 and 95 per cent., the average value of all these F_1 combinations being 73,4 per cent. Though the number of samples taken is sometimes very low, it may be of some interest to cite the average values obtained for each combination. The results were as follows:

F_1 combination	Average value	Number of samples
$C \times D$	74,0 per cent.	17
$C \times E$	76,3 » »	4
$B \times C$	80,0 » »	4
$A \times D$	68,8 » »	4

F_1 combination	Average value	Number of samples
$A \times B$	85,0 per cent.	2
$B \times D$	64,2 » »	5
$B \times E$	76,8 » »	7
$D \times E$	70,0 » »	2

Thus, all the F_1 combinations tested were partially pollen sterile to about the same degree as the parent strains (cf. p. 388—389) and as the hybrids between *A* and *C*. It is not impossible that the hybrids were somewhat more sterile than the parent strains, but the data so far available are not sufficient to prove this assumption.

4. SEED SETTING.

Data on seed setting in the F_1 hybrids were obtained in 1937 and 1938. In the first year seed production in the hybrids between *A* and *C* was measured, the results being those given in Table 4. It is a striking fact that *seed production in the F_1 hybrids was found to be significantly lower than in the parents.* According to the table the number of seeds per spikelet was found to be $1{,}02 \pm 0{,}03$ in the F_1 hybrids, the values of both reciprocal combinations being equal ($1{,}02 \pm 0{,}04$ and $1{,}04 \pm 0{,}07$ respectively). The corresponding values of the parent strains were, after open pollination $1{,}40 \pm 0{,}05$ and $1{,}50 \pm 0{,}05$, and after isolation $1{,}43 \pm 0{,}11$ and $1{,}42 \pm 0{,}16$ respectively. Since the F_1 plants were isolated, their seed production should in the first place be compared with the last-mentioned values. If the variates of both parents are added together into one series, the average value of this series will be $1{,}43 \pm 0{,}09$. This value is significantly higher than the corresponding average of the F_1 plants ($1{,}02 \pm 0{,}03$), the difference being $0{,}41 \pm 0{,}09$ ($D/m = 4{,}28$).

A weakness of the preceding data is the fact that they were obtained from measurements of clonal plants (of parents as well as the F_1 hybrids), using spikes and not plants as units. — The results, however, were confirmed by data from the same cross obtained in 1938 and presented in Table 5. In that year no isolations were made, all the values being obtained from open-pollinated plants. These plants were raised from seeds sown directly in the field. In 1938 seed production in the hybrids was as low as $0{,}95 \pm 0{,}03$. The corresponding value of the *C* parent, $1{,}32 \pm 0{,}03$, is much higher, the difference evidently being significant. As described above, no comparable data were obtained from *A*, due to the accidental breakage of the ears at the harvest of this field.

In field II, however, in which the plants were more widely spaced, values were obtained from both parents. In this field *C* was found to have an average of $1{,}55 \pm 0{,}05$ seeds per spikelet, the corresponding value of *A* being $1{,}49 \pm 0{,}07$. The value of *C* is about the same as the preceding year, and the value of *A* also shows close correspondance. Assuming the same relation between the values for field II, the value of *A* in this field would be 1,27. Undoubtedly this value would have been significantly higher than the average obtained from the F_1 plants.

TABLE 4. *Seed setting in two Triticale strains and their hybrids.*

Strain	Number of seeds per spikelet												n	M ± m
	0,00	0,20	0,40	0,60	0,80	1,00	1,20	1,40	1,60	1,80	2,00	2,20	2,40		
A, isolation.				1	1	1	1	3	3	—	1			11	1,43 ± 0,11
», open pollination...				3	2	8	9	5	9	4	2			42	1,40 ± 0,05
C, isolation.				1	—	1	1	—	—	1	1			5	1,42 ± 0,16
», open pollination...			1	—	—	6	7	12	8	5	1	1		41	1,50 ± 0,05
C × *A*, isolation.....	1	3	1	13	19	18	12	5	4					76	1,02 ± 0,04
A × *C*, isolation.....		2	1	1	9	6	5	4						28	1,04 ± 0,07
Σ F_1, isolation.....	1	5	2	14	28	24	17	9	4					104	1,02 ± 0,03
Σ *P*, isolation.....				2	1	2	2	3	3	1	2			16	1,43 ± 0,09

Thus, taking all data together, it is beyond doubt that the F_1 hybrids in this cross are more sterile than the parents.

In 1938 seed setting was also examined in several other F_1 combinations of the different *Triticale* strains. The average values of the seven combinations tested ranged from 0,58 to 1,22, six of the F_1 generations having values lower than 1,00. These values were much lower than in the corresponding parent strains, the mean values of the parents ranging from 1,17 to 1,55. However, parents and F_1 plants were not strictly comparable, since the parents were grown from seeds sown directly in the field, whereas the F_1 individuals were clonal plants, transplanted to the field in spring. Nevertheless, the great differences observed strongly indicate that also in these crosses fertility is more reduced in the F_1 hybrids than in the parents.

Another observation was made at the same time, viz. that in the hybrids seed setting was very markedly reduced by isolation. As is evident from the following figures, the percentage of seed setting in the isolation bags was much lower than in the open-pollinated ears:

Hybrid combination	Open pollination	Isolation
1. $C \times D$	1,22	0,81
2. $A \times D$	0,58	0,30
3. $C \times B$	1,00	0,43
4. $A \times B$	0,67	0,25
5. $B \times D$	0,58	0,18
6. $B \times E$	0,63	0,20
7. $D \times E$	0,61	0,28

In all the crosses except No. 7 the values are based on counts from both reciprocal combinations.

The very marked reduction in seed setting on isolation is surprising, considering the data presented in Table 4. These data strongly indicate that in the constant strains, *A* and *C*, isolation has little or no effect on seed production. This differential behaviour between the *A* and *C* strains and the F_1 combinations may be due to the fact that, in some cases at least, the F_1 hybrids are more sterile than the constant strains. However, further data must be obtained before definite conclusions can be drawn.

5. MEIOSIS.

So far meiotic observations in F_1 hybrids between the *Triticale* strains have been chiefly confined to $A \times C$, F_1. As reported above (p. 390), the parent strains of this hybrid combination were found to differ significantly as regards the number of univalents present at first metaphase. It is interesting that *the F_1 hybrids were found to be still more irregular than the parents.* According to Table 1 the total average values of *C* and *A* were found to be 1,88 and $6{,}10 \pm 0{,}16$ respectively. On account of the skew distribution in *C* the standard error for this line was not calculated, but it is beyond reasonable doubt that *A* is more irregular than *C*.

In three different slides of F_1 the number of univalents was found to be rather high, the total average value of F_1 being 8,57. Undoubtedly the F_1 hybrids are more irregular than *C*, but in comparison with *A* the difference seems to be less evident. However, if the standard errors of the total series of *A* and F_1 are calculated, the average values to

be compared will be $6{,}10 \pm 0{,}16$ and $8{,}57 \pm 0{,}33$. The difference is $2{,}47 \pm 0{,}37$ and D/m is equal to 6,68. Thus, as far as the observations go, the F_1 hybrids between the *A* and *C* strain have a more irregular meiosis than both parents.

Some other F_1 combinations were also observed to have a rather irregular meiosis. As no comparable fixations of the parent strains were available no detailed counts were made in these hybrids.

III. TRITICALE A × C, F_2.

1. VIGOUR AND FERTILITY.

In 1938 an F_2 material of about 2000 plants was available from the cross $A \times C$ and reciprocally. As already mentioned above, these F_2 plants could be compared with the parents and F_2, which were grown at the same time.

TABLE 5. *Average height, seed setting and kernel quality in the cross Triticale A × C.*

Strain or hybrid generation		*A*, Field II	*C*, Field II	*C*, Field I	F_1, Field I	F_2, Field I
Height	M ± m	147,0 ± 1,5	136,5 ± 1,5	146,8 ± 1,4	161,3 ± 1,3	150,9 ± 0,4
	v	5,8	6,6	7,4	9,2	11,1
	n	30	30	100	128	1946
Number of seeds per spikelet	M ± m	1,49 ± 0,07	1,55 ± 0,05	1,32 ± 0,03	0,95 ± 0,03	0,74 ± 0,01
	v	28,0	18,5	21,9	36,5	60,4
	n	30	30	99	127	1591
Kernel quality	M ± m	4,01 ± 0,23	4,80 ± 0,22	4,25 ± 0,15	3,23 ± 0,11	2,50 ± 0,03
	v	30,8	25,4	26,9	37,0	49,8
	n	30	30	96	126	1580

As is evident from Table 5, F_2 shows a marked decrease in *plant height* in comparison with F_1, the average values being $150{,}9 \pm 0{,}4$ and $161{,}3 \pm 1{,}3$ cm. respectively. The difference, $10{,}4 \pm 1{,}4$, is evidently significant. Since this decrease in plant height gives the impression of inbreeding degeneration, it may be appropriate in this case to give not only the mean values but also the distribution of the variates in F_1 and F_2. The two series were as follows:

	Plant height										
	60	80	100	120	140	160	180	200	220 cm.	n	M ± m
F_1:			1	12	38	72	4	1		128	161,3 ± 1,3
F_2:	7	12	75	317	930	594	11			1956	150,9 ± 0,4

Since the F_1 and F_2 plots were grown side by side the significance of this difference cannot be disputed. The lower average height of F_2 is in good accordance with the high vegetative vigour of F_1 as compared with the parent strains. As is evident from Table 5, the average height of F_2 is only about equal to that of the weaker parent strain ($M = 146{,}8 \pm 1{,}4$).

TABLE 6. *Pollen fertility in the cross Triticale A × C.*

	Percentage of good pollen												
	0	10	20	30	40	50	60	70	80	90	100	n	M
Strain *C*							1	9	7	14	4	35	78,1
» *A*	2	—	1	1	—	—	4	10	22	2	42	74,8
F_1	1	1	—	3	2	6	14	18	20	1	66	69,7
F_2	28	13	10	9	15	22	29	33	42	4	205	57,6

The inbreeding degeneration in F_2 was not only apparent in height but also in fertility. According to Table 6, the average *pollen fertility* shows a decrease from 69,7 in F_1 to 57,6 in F_2. As seen from the distribution of the variates the difference between these values is evidently significant. The distribution in F_2 is still more markedly bimodal than in F_1 and the *A* parent. A considerable proportion of the plants were found to have a very low percentage of good pollen grains.

A similar situation was met with on the female side. *The number of seeds per spikelet* after open pollination showed a great and significant decrease from $0{,}95 \pm 0{,}03$ in F_1 to $0{,}74 \pm 0{,}01$ in F_2 (Table 5). The seed setting might also be expected to show a bimodal distribution, but that was not the case. The F_1 and F_2 series had the following appearance:

	Number of seeds per spikelet											
	0,0—0,2	0,4—0,6	0,8—1,0	1,2—1,4	1,6—1,8	2,0	n	M ± m
F_1:	3	6	10	17	28	32	28	2	—	1	127	$0{,}95 \pm 0{,}03$
F_2:	81	222	313	308	289	215	105	42	14	2	1591	$0{,}74 \pm 0{,}01$

The F_1 and F_2 generations were found to differ not only in the amount of seed setting but also in *the quality of the kernels*. Both parents showed a certain amount of shrivelling of the seeds, which is more marked in *A* than in *C*. The seeds produced by the F_1 plants were found to be inferior to those of both parents, and in F_2 kernel quality was still more reduced. These conditions were measured by gradation,

using a scale from 1 to 10. The higher values represent the better qualities. As may be seen from Table 5, the average value of F_1 was found to be $3{,}23 \pm 0{,}11$, this value being lower than those of the parent strains. The F_2 average, $2{,}50 \pm 0{,}03$, shows a considerable and significant decrease in comparison with F_1 and the parents.

Before leaving Table 5, it should be pointed out that the coefficients of variation are higher in F_2 than in F_1 and the parent strains. This must be interpreted as being a consequence of segregation, affecting vigour, as well as fertility and quality of the seeds.

2. CORRELATIONS.

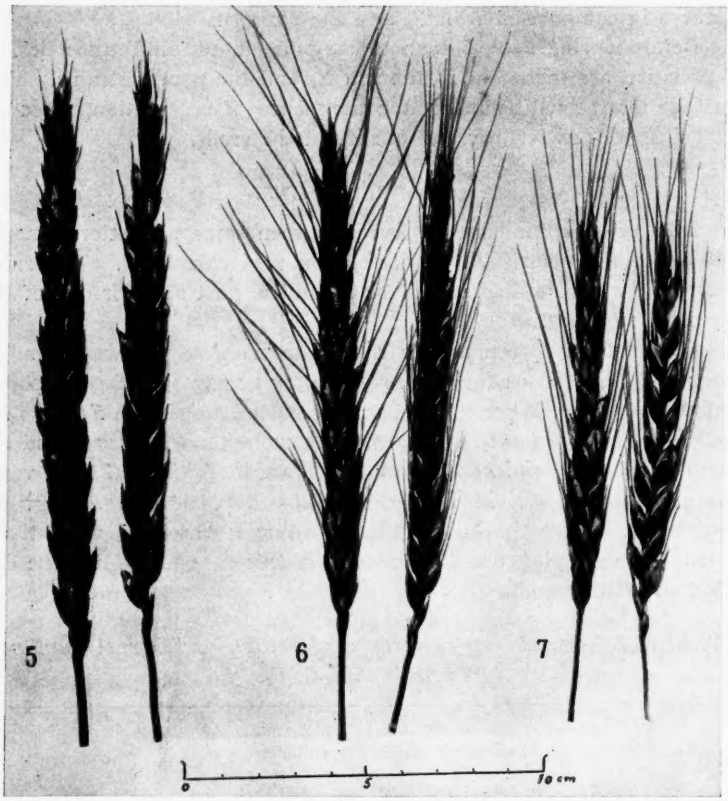
In F_2 correlation was observed between some characters, and in such cases the coefficient of correlation was calculated. The same coefficient was also determined for F_1 and in part also for one of the parents, *Triticale C*.

Correlation between *pollen fertility and seed setting* was found to occur in F_1 as well as F_2. The *r* value for percentage of good pollen and number of seeds per spikelet was found to be $+ 0{,}39$ in F_1 ($n = 58$) and $+ 0{,}42$ in F_2 ($n = 67$). — Positive correlation was also found between *seed setting and kernel quality*. Thus, in *Triticale C* the *r* value was $+ 0{,}58$ ($n = 96$), in $F_1 = + 0{,}64$ ($n = 126$) and in $F_2 = + 0{,}78$ ($n = 1572$). Since the last-mentioned value is based on a relatively high number of plants it is of interest in this case to publish the distribution of the variates.

TABLE 7. *Correlation between seed setting and kernel quality, Triticale A × C, F_2.*

Quality	Number of seeds per spikelet											n	M
	0,0	0,2	0,4	0,6	0,8	1,0	1,2	1,4	1,6	1,8	2,0		
10												—	—
9								1				1	(1,30)
8									1			1	(1,50)
7						2	3	3	5	1	2	16	1,38
6			1	—	—	—	3	11	7	6		28	1,38
5			1	—	3	6	14	16	13	4		57	1,22
4			3	13	24	49	59	32	6	1		187	0,99
3		6	21	55	81	105	87	33	7	1		396	0,85
2		20	91	150	146	104	44	9	2	1		567	0,63
1		38	105	94	54	22	5	—	1	—		319	0,46

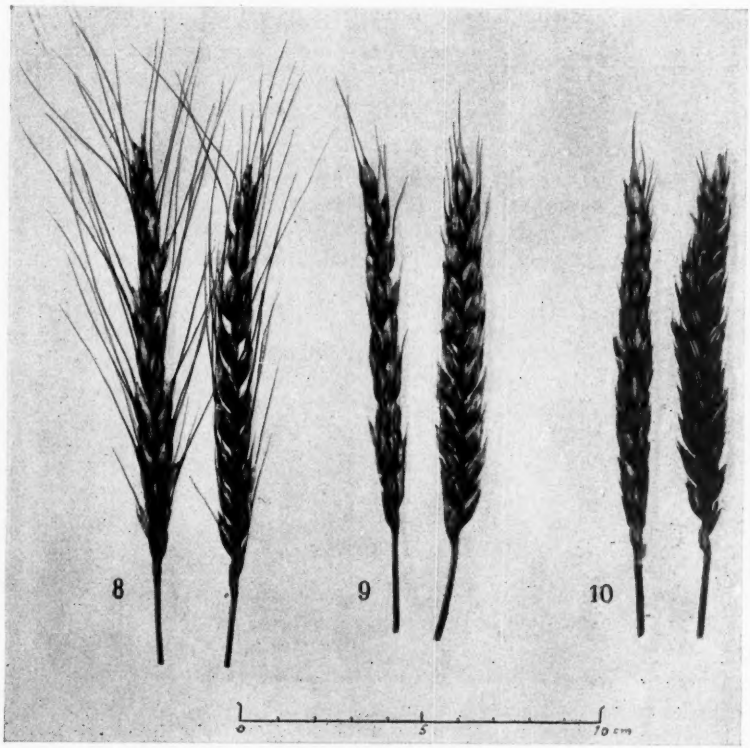
As is evident from Table 7, the number of seeds per spikelet increases continuously from 0,46 to 1,38 as the kernel quality improves from 1 to 7.



Figs. 5—7. Ears of three of the six constant *Triticale* strains studied. — Fig. 5, *Triticale A*; Fig. 6, *Triticale B*; Fig. 7, *Triticale C*. — Figs. 5 and 6 represent the types produced by RIMPAU, Fig. 7 is of Russian origin. The cross combination most extensively studied is that between strains *A* and *C* (Figs. 5 and 7).

A third positive correlation was observed to occur, viz. between *degree of tillering and seed setting*. The vigorous plants with many shoots were on an average observed to have a higher number of seeds per spikelet than those having a low number of shoots. The *r* values were found to be + 0,47 in strain *C* (n = 39), + 0,37 in F_1 (n = 102)

and + 0,32 in F_2 (n = 1592). It may be of interest to cite the average seed setting in F_2 in the different classes of tillering. These values were as follows:



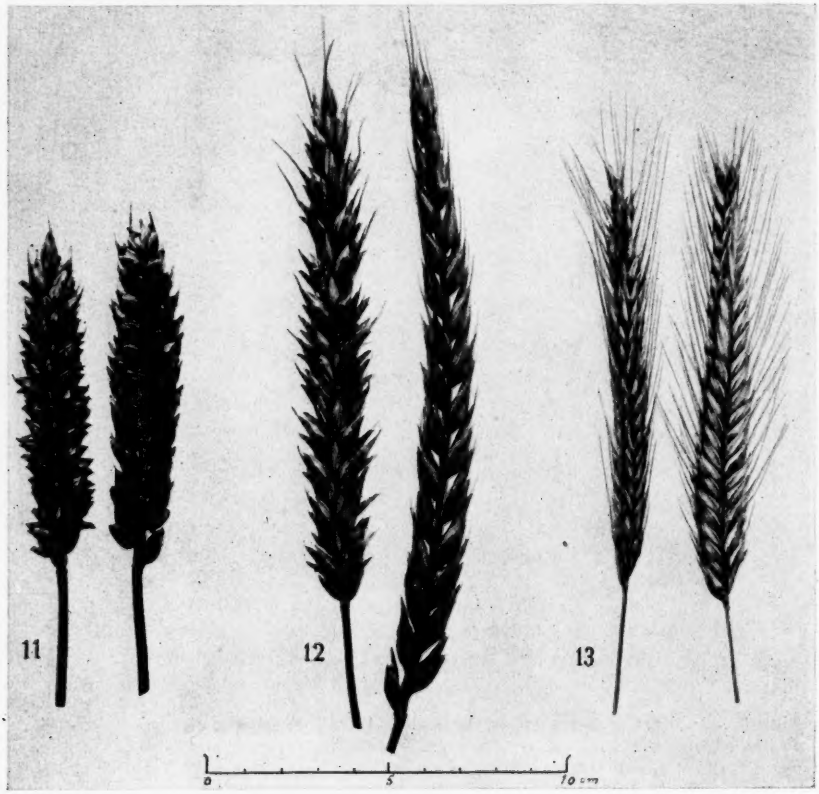
Figs. 8—10. Ears of three of the six constant *Triticale* strains studied. — Fig. 8, *Triticale D*; Fig. 9, *Triticale E*; Fig. 10, *Triticale F*. — Fig. 8 is of Russian origin, Fig. 9 is the strain of TAYLOR and QUISENBERRY and Fig. 10 represents *Triticale* Svalöf (a combination of »Solvete III» and »Midsommarråg»).

	Number of shoots						
	0 — 3	— 6	— 9	— 12	— 15	— 18	— 21
Average number of seeds per spikelet:	0,64	0,71	0,95	0,98	1,15	1,60	1,45
Number of individuals:	436	792	267	67	22	4	4

Though the coefficient of correlation is rather low (+ 0,32), there is evidently a continuous increase in the number of seeds per spikelet as the shoot number increases from 1 to 14.

IV. THE PRODUCTION OF NEW HETEROZYGOTES WITH 56 CHROMOSOMES.

In addition to crossing and recombination of different *Triticale* strains already existing, some other methods may be successful in



Figs. 11—13. Ears of a new *Triticale* product (Fig. 12) in comparison with ears of wheat (Fig. 11) and rye (Fig. 13). — Fig. 11 represents »Solvete III»; Fig. 13, »Stålråg» and Fig. 12 two different *Triticale* plants from the I_2 generation of a new *Triticale* heterozygote. This heterozygote arose from pollination of a hybrid between Swedish wheat and rye with *Triticale* pollen. The large ear and kernel dimensions are conspicuous. — Figs. 11—13 are directly comparable to Figs. 5—10.

producing new rye—wheat amphidiploids. The following different ways have been tried with more or less success.

1. POLLINATION OF PRIMARY WHEAT—RYE HYBRIDS WITH TRITICALE POLLEN.

A. CHROMOSOMAL VARIATION.

Since the primary hybrids between wheat and rye are known to produce a certain proportion of unreduced ovules, and since the union of gametes having the same chromosome number generally gives a better result than the union of gametes with different chromosome numbers, the primary wheat—rye hybrids have been exposed to *Triticale* pollen. The union of unreduced ovules with 28 chromosomes and reduced *Triticale* pollen grains with the same chromosome number should give new *Triticale* plants with 56 chromosomes. Such results were, in fact, obtained in experiments of this kind performed in the years 1936—1938.

Most of the seeds harvested were from open-pollinated flowers, and only a minority was produced by direct crosses. However, in order to secure wind pollination of the kind intended the wheat—rye F_1 hybrids were surrounded by *Triticale* plants (hitherto exclusively *A* and *C* plants). As the *Triticale* plants flower more openly than wheat, a certain amount of wind pollination was indeed effected. For this work it was found to be very useful to increase the number of F_1 hybrids in the manner described by RIEBESEL (1937).

The seeds harvested on the primary wheat—rye hybrids with 28 chromosomes gave rise to a total of 201 daughter plants. The chromosome numbers of these plants were determined and the results of these counts are given in Table 8.

According to the table, the chromosome numbers vary from 35 to 69, all numbers between 35 and 60 being represented besides the single plant with $2n = 69$. The total distribution is clearly bimodal, one maximum being situated at 49, the other one at 56. The former maximum is represented by 11 per cent. of the plants, the second maximum by not less than 23 per cent. Undoubtedly these maxima correspond to the union of unreduced ovules with male gametes, containing either 21 or 28 chromosomes. These pollen grains certainly originated from ordinary wheat and *Triticale* plants respectively. This is especially evident in a comparison between the results in 1937 and 1938. In the first year the maximum at 49 is much larger than at 56, in the second year the maximum at 49 has almost vanished, whereas the maximum at 56 is quite conspicuous. The explanation of this difference between the results of 1937 and 1938 is simply that in 1937 the

TABLE 8. *Chromosome numbers in the progenies of wheat × rye, F_1 hybrids.*

Year	Somatic chromosome number																											n
	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	69	
1936													1	—	2	—	—	—	—	1	1	2						7
1937		1	1	—	3	2	1	4	2	1	1	4	7	8	15	2	1	3	2	4	1	9	1					73
1938	1	2	2	4	1	—	1	—	1	—	2	1	2	4	5	3	2	2	10	14	20	36	1	4	1	1	1	121
Total	1	3	3	4	4	2	2	4	3	1	3	5	10	12	22	5	3	5	12	19	22	47	2	4	1	1	1	201

TABLE 9. *Chromosomal variation in the progeny of wheat × rye hybrids, involving different wheat varieties.*

Year	F_1 combination	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	69	n	Percentage of plants with at least 47 chromosomes
1937	Sol III × Stålråg						1	—	—	—	—	—	1	1	2	6	2	1	—	—	—	—	3						17	88,2 ± 7,8
»	Standard × »					2	1	—	2	2	—	—	2	3	3	2	—	—	1	2	4	1	5						30	70,0 ± 8,4
1938	Sol III × »							1	—	—	—	1	1	2	3	4	3	1	1	9	12	19	34	1	4	1	1	1	99	97,0 ± 1,7
»	Standard × »	1	2	2	5	1	—	—	—	1	—	1	—	—	1	1	—	1	1	1	4	1	2						25	48,0 ± 10,0

hybrids were grown not far from large wheat fields, whereas in 1938 they were grown in a more isolated field. Thus in the last-mentioned year practically only *Triticale* pollen was available for the F_1 plants, but in 1937 this kind of pollen had to compete with the pollen from the neighbouring wheat fields.

Another rather striking fact to be seen from the table is that the unreduced ovules may not only have the exact somatic number 28 but evidently also slightly lower or slightly higher numbers. This will account for the formation of the plants with 52—55 and 57—60 chromosomes. In several of the plants with more than 56 chromosomes the counts were especially controlled and were found to be correct. The plant with 69 chromosomes is remarkable, and at present it is difficult to give a plausible explanation of its origin.

For the production of the primary hybrids crosses were made between selected Swedish commercial varieties of wheat and rye. The rye variety predominantly used was »Stålråg». Of the wheat varieties »Sol III» and »Standard» have played the greatest rôle in the crosses. Greatly to our surprise it was found that the F_1 hybrids with »Sol III» produce descendants with 56 chromosomes in a higher frequency than the hybrids with the other wheat variety »Standard». This rather unexpected result is seen in Table 9.

Both wheat varieties were used for crosses in 1936 and 1937 and in both years they were pollinated with the same rye variety, »Stålråg». The resulting F_1 hybrids gave a total of 171 descendants, the chromosome numbers of which were determined in 1937 and 1938. Somewhat arbitrarily the material may be divided into two groups consisting of plants with 47 or more chromosomes and those having numbers lower than 47. The plants in the former group may be regarded as the result of a union between male gametes with 21 or 28 chromosomes and unreduced or approximately unreduced ovules. In 1937 $88{,}2 \pm 7{,}8$ per cent. of the descendants of the Sol III $\times$ Stålråg hybrids had high chromosome numbers, the corresponding value of the Standard $\times$ Stålråg hybrids being $70{,}0 \pm 8{,}4$. The probability that the difference between these values is significant is not very great, but in the following year definite results in this respect were obtained. In 1938 the hybrids with Sol III gave $97{,}0 \pm 1{,}7$ per cent. descendants with high chromosome numbers, the corresponding value of Standard being as low as $48{,}0 \pm 10{,}0$. The difference $97{,}0 - 48{,}0$ is $49{,}0 \pm 10{,}0$ and is thus of significance.

This difference in average chromosome number of the progenies

is correlated with a clear difference in fertility of the mother plants. In 1938 a total of 125 seeds were harvested from 389 ears of Sol III × Stålråg hybrids, the corresponding value of the Standard × Stålråg hybrids being only 28 seeds from 1143 ears. The significance of this difference is immediately apparent.

The differential behaviour of Sol III and Standard is interesting but difficult to explain. The only possible explanation seems to be that the genotypic constitution of Sol III causes the formation of a larger proportion of unreduced gametes in the hybrids with rye than is the case with Standard. This would also explain the better fertility of the Sol III hybrids, since unreduced ovules will give more viable zygotes with wheat or *Triticale* pollen than reduced ovules.

Similar conditions seem to occur in the material studied by VON BERG and OEHLER (1938). According to these authors (l. c. p. 232) special F_1 families gave much better results when pollinated with *Triticale* RIMPAU than other families.

B. FERTILITY AND VIGOUR OF THE PLANTS OBTAINED.

The data as yet available concerning the properties of the descendants of $F_1 \times$ *Triticale* crosses are fragmentary, but nevertheless they are sufficient to demonstrate that plants with 56 chromosomes are superior to those having lower, aneuploid numbers.

Thus, for instance, *pollen fertility* was observed to have the following values in 18 plants tested:

	Percentage of apparently good grains												
	0	10	20	30	40	50	60	70	80	90	100	n	M
Plants with 55—57 chromosomes				1	—	1	—	—	2	4		8	70,0
Plants with lower numbers	1	3	1	1	2	1	1					10	32,0

The wide difference between the average values strongly indicates that exactly or approximately octoploid plants have better pollen fertility than the other plants. That the distribution of the two series is really significantly different is evident from a χ^2 test. If the series are divided into two parts with 50 per cent. as a limit, the $\Sigma\chi^2$ will be 5,42 and P less than 0,02.

Seed setting is evidently influenced by the chromosome number in the same way as pollen fertility. In 1937 5 plants of the kind discussed were in culture. Three of these had ± 56 chromosomes, the other two

were deviating in chromosome number ($2n = \pm 54$ and ± 47 respectively). In the former plants the average number of seeds per ear was found to be 14,3 (21 ears, 300 seeds). In the latter two plants the corresponding value was 2,0 (8 ears, 16 seeds). In the next year the observations made pointed in the same direction. Eight plants with 56 chromosomes had an average of 0,95 seeds per spikelet, the corresponding value of five plants with 54—55 chromosomes being 0,65. These values were from open-pollinated ears.

Eleven of the plants were also isolated, and without exception the degree of seed setting was lower in the isolation bags than in the open-pollinated ears. The total average values obtained for these plants were 0,98 seeds per spikelet after open pollination and 0,52 after isolation. Thus, as regards the reaction to isolation the new *Triticale* heterozygotes, obtained from the crosses $F_1 \times$ *Triticale*, behave in the same way as the F_1 hybrids between different *Triticale* strains (cf. above p. 404).

Plant height is probably also influenced by chromosome number in the same way as fertility. In 1938 twenty-nine plants were measured, and of these 11 plants with 55—57 chromosomes had an average height of 114,1 cm. The corresponding value of 18 plants with lower chromosome numbers was 100,6. A χ^2 test gave the result that the two series in question were different with a probability of approximately 6 : 1.

2. CROSSES BETWEEN TRITICALE AND TRITICUM VULGARE.

As a third method of producing new *Triticale* strains crosses were undertaken between some *Triticale strains* and *Triticum vulgare*. In the offspring of the primary hybrids with 49 chromosomes new types with 56 chromosomes may be selected. The results obtained during this work may be summarized as follows:

A. CHROMOSOMAL VARIATION.

After crosses between *Triticale C* and Swedish commercial varieties of *Triticum vulgare* (chiefly »Standard») seven F_1 plants were obtained in the following year. Of these F_1 plants four had the expected 49 chromosomes, the remaining three having the chromosome numbers ± 47, ± 51 and 52 respectively. Since the crosses were made with *Triticale C* as the female parent, it is obvious that in this strain a proportion of functional ovules with somewhat deviating chromosome number are formed. This is not surprising, considering the meiotic irregularities observed in the pollen mother cells of this strain. From the seven F_1 plants progenies were raised, partly after isolation, partly

TABLE 10. *Chromosomal variation in F_2 progenies of crosses between Triticale and Triticum vulgare.*

Hybrid combination	Field number	Chromosome numbers of the F_1 plants	Chromosome numbers in the F_2 progenies 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56	n	M
Triticale C × Triticum vulgare	36—32—2, isolated	52	1 2 1	4	52,0
	—4, open pollin.	49	1 1	2	45,5
	—5, isolated .....)	49	2 1 1 — 2	6	44,8
	—5, open pollin. .....)	»	1	1	43,0
	—6, isolated	± 51	1 6 10 5 5 1 1 — — 1	30	44,7
	—7, » .....)	49	1	1	43,0
	—7, open pollin. .....)	»	1 1 2 1	5	45,6
	—9, isolated	49	1 4 9 16 12 3 2 1 — — — 1 — — 1	50	45,5
	36—33—1, »	± 47	1 1 — 3 1	6	44,3
	Total [1] (isol. + open poll.)	47—51	3 15 21 27 21 7 3 1 — 1 — 1 — — 1	101	45,13
	» (isolated)	» »	3 14 20 25 18 6 3 1 — 1 — 1 — — 1	93	45,12 ± 0,22
A × vulgare	37—33, isolated	± 49 and ± 50	3 7 12 11 22 15 8 8 4 1 2	93	47,22 ± 0,23

[1] 36—32—2 excepted.

TABLE 11. *Chromosomal variation in F_3 progenies of Triticale × Triticum vulgare.*

Cross combination	Field number	Chromosome numbers of the mother plants	Chromosome numbers in the F_3 progenies 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62	n	M
C × vulgare	1938—66, open pollin.	± 51	3 18 20 24 16 10 2 1	94	45,80
»	69, isolation .....)	53	1 — — 3 6 5 — 2 — 1	18	51,50
»	70, open pollin.)	»	1 1	2	(51,50)
»	67, isolation .....)	± 56	1 — — — 1 2 3 14 14 11 10 — 3	59	56,08
»	68, open pollin.)	»	1 — — — 1 5 11 10 6 6 5 2 — 1	48	56,33
A × vulgare	1939—56 » »	50—52	1 6 4 3 7 9 4 3 — — 1	38	46,97

after open pollination. The chromosome numbers of 101 F_2 plants were determined with the results shown in Table 10.

The main result to be seen from the table is the fact that *almost all the F_2 plants have lower chromosome numbers than the mother individuals*, and among the 101 F_2 individuals there is only one single octoploid with 56 chromosomes. The four descendants of the F_1 plants with 52 chromosomes are exceptional by keeping up their average chromosome number to the same level as the value found in their mother. All the other progenies behave in about the same way and show an average decrease of the chromosome number from $\pm$ 49 to 45, approximately. This decrease is probably caused to a great extent by chromosome elimination at meiosis in the F_1 hybrids.

Analogous crosses were also undertaken with another *Triticale* strain, *A*, this strain as the mother being hybridized with the commercial wheat variety »Drottningvete». Two F_1 hybrids were obtained, and these plants were found to have $\pm$ 49 and $\pm$ 50 chromosomes respectively. In F_2 the chromosome numbers of 93 individuals were determined and found to vary between 43 and 53 (Table 10, below). In this case not a single F_2 plant with 56 chromosomes was produced, but on an average this F_2 generation kept up the chromosome number much better than the F_2 progenies of $C \times$ *vulgare*. All the F_2 plants of $A \times$ *vulgare* were from isolated F_1 ears and their average chromosome number was $47{,}22 \pm 0{,}23$. As may be seen in the table, the corresponding value of $C \times$ *vulgare* (only progenies after isolation) was only $45{,}12 \pm 0{,}22$. The difference between these values, $2{,}10 \pm 0{,}32$, is significant. The causes underlying this difference are not known but the most plausible hypothesis seems to be a higher rate of chromosomal elimination in the $C \times$ *vulgare*, than in the $A \times$ *vulgare* hybrids. It is strange, however, that strain *A* should give more regular F_1 hybrids than strain *C*, since the *A* strain itself has a more irregular meiosis than *C*. It is quite possible, too, that the different wheat varieties used may be responsible for the result. Finally, it should be mentioned that the two F_2 generations were grown in different years, and thus it is possible that different environmental conditions have influenced the rate of elimination.

From both the crosses discussed above further generations have been raised, and at the time of writing F_4 progenies of $C \times$ *vulgare* and F_3 progenies of $A \times$ *vulgare* are flowering. Chromosome counts in F_3 progenies of both crosses were undertaken, the results being summarized in Table 11.

As may be seen from the table, three different progenies were raised from three F_2 plants of $C \times$ *vulgare*, having the chromosome numbers (±) 51, 53 and (±) 56 respectively. The progeny from the first of these plants shows a very marked decrease in chromosome number, all the 94 plants examined having lower numbers than the mother individual. The average value of this progeny is 45,80. Without further selection, and even if selection were tried, this material would certainly soon revert to the hexaploid number 42.

In the next progeny, from the F_2 plant with 53 chromosomes, the average decrease is less marked and amounts to only 1,50 instead of 5,20 in the case just discussed. In this second progeny a few plants had higher chromosome numbers than the mother, one of them even reaching the value of 56.

A rather striking contrast to the former two progenies is represented by the descendants of the F_2 plant with $2n = 56$. As is evident from the table, there is in this case on an average no decrease in chromosome number, the mean value after isolation being 56,08 and after open pollination 56,33. Being based on a total of 107 plants these values are quite reliable. It is striking, however, that in these progenies chromosome variation is very great, the chromosome numbers ranging from 48 to 62. Thus, *in this material the chromosome number oscillates greatly, but on the average the octoploid number is kept up.*

The F_3 progeny of $A \times$ *vulgare* was not raised from a single F_2 plant, but the seeds of all F_2 plants with at least 50 chromosomes were mixed [1], and the mixture sown directly in the field. Fifty seeds, however, were germinated in pots and the chromosome numbers of the resulting 38 plants were determined with the result shown in Table 11. Evidently the great majority of these plants have less than 50 chromosomes, the average value being 46,97. Thus, also in this case, as in the similar progenies of $C \times$ *vulgare*, F_3, there is a strong tendency to revert to the hexaploid number 42.

Nevertheless, the production of a new ± octoploid *Triticale* strain from the cross $C \times$ *vulgare* demonstrates that it is really possible in this way to produce new *Triticale* strains. This method, however, can hardly be recommended in comparison with the two other methods. It should also be observed that by *Triticale* $\times$ *vulgare* crosses only the

[1] When this mixture was made the F_2 plants with 53 chromosomes had not yet been detected. Thus, the mother plants of this F_3 progeny had chiefly 50 and 51 chromosomes.

wheat component in *Triticale* may be essentially changed, no new rye chromosomes being introduced.

B. CORRELATION BETWEEN CHROMOSOME NUMBER AND VIGOUR.

In F_2 and F_3 progenies of $C \times$ *vulgare* and $A \times$ *vulgare* height measurements were made. These height values, as an expression of vigour, were correlated with the chromosome numbers, the following results being obtained (Table 12).

The table is based on the sum of the height values of $C \times$ *vulgare*, F_2 and $A \times$ *vulgare*, F_2. In order to allow summation, the height figures

TABLE 12. *Correlation between chromosome number and plant height; Triticale* $\times$ *Triticum vulgare*, F_2.

Chromosome number	Plant height													n	M ± m
	30	40	50	60	70	80	90	100	110	120	130	140	150		
42—43					1	1	3	7	1	2	3	1		19	110,3 ± 5,8
44—45				1	1	13	17	11	10	1	3			57	99,9 ± 3,3
46—47	1	—	1	1	1	7	7	10	11	2				41	98,9 ± 3,9
48—49				3	1	6	1	3	1					15	87,0 ± 6,5
50—51					1	1	1	1	—	1	1			6	103,3 ± 10,2
52—53						1	—	1						2	95,0 ± 17,7
54—55														—	— —
56—57										1				1	115,0 ± 25,1

are relative values, *i. e.* they are expressed in percentage of the average values of the respective F_2 progenies. — According to the table, *height shows a continuous decrease as the chromosome numbers increase from 42 to 49.* The most clear difference is between 110,3 ± 5,8 in the class 42—43 and 87,0 ± 6,5 in the class 48—49. The difference is 2,33 ± 0,87 and D/m = 2,68. The odds of this difference being significant are 135 to 1. — Plants with more than 49 chromosomes seem to have better vigour than the most intermediate class, but the individuals are too few to allow any safe conclusion.

Correlation between chromosome number and plant height was also studied in that F_3 progeny of $C \times$ *vulgare* which was derived from the single F_2 plant with 2n = 56. In this progeny the average height in the classes 54—58 was the following:

Chromosome number	Average height	Number of plants
54	103,6 cm.	7
55	102,6 »	25
56	103,7 »	23
57	97,9 »	14
58	93,0 »	15

Though no significant differences were found between these average values, it may be concluded, however, that the plants with exactly 56 chromosomes are at least as vigorous as the plants with slightly aberrant numbers.

TABLE 13. *Correlation between chromosome number and seed setting; Triticale A × Triticum vulgare, F_2.*

Chromosome number	Number of seeds per spikelet											n	M
	0,0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1,0		
42—43		1	—	1	—	—	—	—	1			3	0,40
44—45	5	—	3	1	2	1	3	—	—	—	1	16	0,31
46—47	13	3	1	2	6	1	2	—	1			29	0,21
48—49	13	3	1	2	1							20	0,08
50—51	3	—	—	2	2	—	—	1	—	1	1	10	0,40
52—53				2								2	0,30

In the same progeny the following fragmentary values were also available on *seed setting* after open pollination.

Chromosome number	Average number of seeds per spikelet	Number of plants
55	0,61	9
56	0,62	10
57	0,55	8
58	0,50	7

If these data are considered in combination with the height values just mentioned, it seems probable that the plants with 56 chromosomes are really somewhat more successful than the surrounding aneuploids.

More extensive data on seed setting in the crosses *Triticale × Triticum vulgare* were obtained from *Rimpau × vulgare*, F_2. In 80 F_2 plants the above correlation was observed between chromosome number and seed setting after open pollination (Table 13). Just as in the case of plant height (Table 12) there is a rather obvious decrease in seed

setting as the chromosome number increases from 42 to 49. Still higher chromosome numbers seem to improve seed setting again.

V. DISCUSSION.

From the data accumulated it is evident that the *Triticale* strains differ in many respects. These differences apply not only to morphological characters, plant height and general vigour but also to physiological and chemical properties, male and female fertility and mode of meiosis. Similar results were obtained by VON BERG and OEHLER (1938) and VON ROSENSTIEL (1938). VON BERG and OEHLER observed significant differences in seed setting, VON ROSENSTIEL differences in resistance to brown rust.

Thus, it is not possible to bring the characteristics of rye-wheat into one formula. It should rather be stressed that in the first place the properties of *Triticale* plants is a question of biotype. Though rather self-evident, knowing the inumerable differences between wheat as well as rye varieties, this clear differentiation of the *Triticale* material is interesting from the theoretical as well as the practical point of view.

As is well known, experimental polyploids often differ from natural polyploids in having a more or less reduced fertility. Sometimes also viability in the experimental products seems to be insufficient to stand the test of natural selection. However, when a new experimental polyploid is composed not only of a single new biotype but, as in the present case, of a number of strains with quite different properties, it is much easier to understand how new successful polyploids may arise in nature. Thus, supposing that *Triticale* were a new wild allopolyploid species subject to natural selection, its chances of survival would depend very much on the constitution of the biotypes representing the new species. Strain *F* in our material would certainly not be successful, since it is inferior in vigour as well as in fertility. Strains *A* and *C* would be better, the first because of its very good vigour, the second on account of its relatively good fertility. A biotype combining the different advantages of *A* and *C*, finally, would certainly have the best chances of survival. Such a biotype might, indeed, be formed by natural crossing.

In our experiments strain *A* and *C* were crossed with a practical purpose in view, and besides this combination several other crosses have been made. This means the beginning of a rye-wheat breeding by means of recombination. Recombination, however, can only lead to new and improved varieties, if the parent strains are different. Since

such differences really occur, it may be safely concluded that the production of new *Triticale* types, which are superior to the parent strains, is only a question of time. It is quite a different matter, however, whether these new improved varieties will ever be good enough to be of practical importance. Preliminary field trials with strain *C*, performed by Prof. ÅKERMAN, have shown that this rye-wheat type gives only about 60—65 per cent. of the yield obtained from the best commercial varieties of wheat. — It should be remembered, however, that these high-yielding wheat varieties are the result of intensive breeding work carried on for several decades. It remains to be seen how much can be reached by a similar amount of *Triticale* breeding.

The first cross between different *Triticale* strains made by the present writer is now in F_3, this generation consisting of 72 families. The mother plants of these F_3 families were selected from about 2000 F_2 plants, the main basis of selection being the number of seeds per spikelet. The F_3 families now growing in the field represent a multitude of different types, several of them being relatively stabilized. Differences in vigour are quite striking, differences in fertility are expected to be observed after the harvest. It should be noted that the selection work will be facilitated by the fact that high yield and good kernel quality were found to be positively correlated, the r value in F_2 being $+ 0{,}78$. Thus selection of high-yielding families in F_3 and following generations will at the same time improve the average kernel quality.

Another positive correlation of practical interest is that between seed setting and degree of tillering, the vigorous plants with many shoots being more fertile than the weaker plants. As reported above, the r value was found to be $+ 0{,}32$ in F_2.

The cytological lability is an important characteristic of *Triticale*, unpaired chromosomes at I—M having been observed by several workers. Since this meiotic imperfection is evidently correlated with the different degrees of partial sterility observed in all *Triticale* strains, it deserves careful attention. It is highly probable that non-conjunction in the *Triticale* strains represents essentially the same phenomenon as the decrease in chromosome pairing observed by LAMM (1936) in inbred lines of rye. According to his investigations the inbred lines (I_7—I_9) showed an average reduction in chiasma number, which to a certain extent was accompanied by non-conjunction. The degree of these disturbances, however, were different in different lines. In this connection the results of VON BERG and OEHLER (1938) are interesting. According

to these authors the chiasma frequency in some *Triticale* strains was found to be somewhat lower than in *Triticum vulgare*.

Already from their good uniformity and constancy (*Triticale* RIMPAU has bred true to type for almost 50 years) it is evident that the *Triticale* strains are self-fertilizing. In the main this was verified by the isolation experiments undertaken, though some hybrids of various kinds showed a decreased seed production in the isolation bags. Since *Triticale* represents a synthesis of wheat, which is a typical self-fertilizer, and rye, which is pronouncedly allogamous, the normal self-fertilization in *Triticale* must lead to an automatic inbreeding degeneration of the rye component. This partial inbreeding degeneration is probably the main cause of the meiotic lability, and the reduced fertility of *Triticale*. It is probably also responsible for the rather poor vigour of some *Triticale* strains.

Under such circumstances it seems desirable, in future breeding work with *Triticale*, to use selected inbred lines of rye for the crosses instead of population rye. In ordinary rye populations, as in allogamous species in general, the gametes are probably very different as regards the factors influencing viability and fertility. That such is the case is evident from our experience with haploids of rye, timothy and *Dactylis glomerata*. These species are all allogamous, and in each species different haploids have been found to represent different degrees of viability. In *Dactylis* they were also found to have different degrees of fertility. Thus, the fate of a *Triticale* strain will largely be determined by the constitution of the rye pollen grain, which happens to function in the primary cross.

At this institute we have a series of inbred lines of rye at our disposal (material raised by Prof. H. NILSSON-EHLE) which may prove to be very valuable for the *Triticale* breeding. Some of these lines, which have been inbred for 15 years, combine complete uniformity with a relatively good vigour and self-fertility. Such lines are now exclusively used for the crosses. — In general it seems necessary to make a thorough investigation of viability, meiotic behaviour and fertility in the inbred rye lines in order to obtain a better understanding of the properties of *Triticale* strains. This is necessary since *Triticale* »*vulgare-cereale*» does not represent a combination of wheat and ordinary rye but a combination of wheat and inbred rye.

The cytological lability in *Triticale* may be regarded as being due to physiological disturbances, which through the action of meiotic irregularities are also responsible for the reduction in fertility. The

correlation present between sterility and chromosomal irregularities is illustrated best by the behaviour of the F_1 hybrids between strains *A* and *C*. This hybrid combination seems to have a higher frequency of univalents than both parent strains and was also found to be significantly more sterile than the parents. It is doubtful, however, whether all sterility in this material is directly caused by meiotic irregularities. It is possible that sterility and meiotic irregularities may only be two different symptoms of the same physiological disturbance. The two interpretations may be clarified in the following way:

Interpretation 1: physiological disturbance → meiotic irregularity → → partial sterility.

Interpretation 2: physiological disturbance → partial sterility
↘ meiotic irregularity

According to the first alternative sterility should be exclusively haplontic, according to the second alternative exclusively diplontic. The truth is probably a combination of both interpretations. It is clear, indeed, that the rather high frequency of univalents at meiosis must lead to haplontic sterility, but some observations also indicate that interpretation 2 may be correct. Thus, strain *A* is cytologically much more labile than *C*, but in spite of this the two strains have a rather equal degree of seed setting. It is true, however, that kernel quality in *A* is less good than in *C*. — Secondly it has been observed that in strains *A* and *F* seed setting is often less good in the uppermost part of the ears than in the lower part. In strain *F* the tip of the ear may be almost defective and in such cases sterile. It seems clear that this sterility is caused by direct somatic influences and not indirectly by the action of meiotic irregularities. The same view is supported by the fact that the number of seeds per spikelet was found to be positively correlated with plant vigour, as measured by the number of shoots per plant (cf. p. 408).

In the two strains studied, *A* and *C*, the meiotic irregularities almost exclusively consist of the occurrence of non-conjunction. Otherwise the chromosomes quite predominantly form normal bivalents. This was especially apparent in strain *C*, in which approximately half of the pollen mother cells undergo a fairly regular meiosis without visible disturbances. These results show that the true *Triticale* strains with 56 chromosomes behave in quite a different way from that of derivatives of wheat-rye hybrids with lower numbers. KATTERMANN (1935) made a detailed study of the chromosome associations in 47 daughter plants

of open-pollinated wheat-rye hybrids. The chromosome numbers in this material varied from 38 to 52 and at meiosis multivalent associations were found to be quite frequent. According to KATTERMANN (l. c.) formation of multivalents was frequent also in plants having complete wheat genomes. In such plants the »differential affinity» should be expected to bring about a regular pairing of the wheat chromosomes, resulting in 21 bivalents. However, the presence of extra rye chromosomes evidently counteracts the differential affinity and leads to various deviating kinds of association. Regular pairing may occur only when the plant possesses a complete set of the rye as well as the wheat chromosomes.

If the incomplete chromosome association in *Triticale* is an inbreeding phenomenon, the frequency of univalents might be expected to be lower in F_1 hybrids between different *Triticale* strains. By the cross two different rye genomes will be brought together, and since chromosome association was found to be better in population plants than in inbred plants of rye (LAMM, 1936) the *Triticale* hybrids might also be expected to be more regular than the constant and partially inbred *Triticale* strains. This was not found to be true, however, the result being rather in the opposite direction. In the only hybrids sufficiently studied so far, those between *A* and *C*, the number of univalents was found to be higher than in both parents (Table 1). Also in newly arisen octoploid plants from the cross $F_1 \times$ *Triticale* preliminary observations indicate a rather high frequency of unpaired chromosomes.

In another respect, however, hybridization of *Triticale* strains conforms to expectation. The F_1 hybrids were found to be vigorous, and in one case at least decidedly more vigorous than the average vigour of the parent strains. This agrees very well with the fact that crosses between inbred rye lines result in vigorous F_1 hybrids. It is also in accordance to expectation that in the *Triticale* cross $A \times C$ there is a marked decrease in vigour from F_1 to F_2. According to Table 5 and the height distributions given on page 405, the average height of F_2 was found to be significantly lower than that of F_1. This may be due to the automatic self-pollination of F_1, the F_2 generation thus being inbred. It should also be borne in mind, however, that meiosis in F_1 was found to be more irregular than in the parents. This will probably cause an increased frequency of aberrants in F_2, these aberrants being less vigorous than the euploid plants. This may also explain the marked decrease in fertility observed and the appearance of a special group of highly sterile F_2 plants.

Several other data obtained demonstrate that the octoploid condition really represents a state of balance, which is separated from the hexaploid condition by a zone of inferior viability and fertility. The height measurements and studies of pollen fertility and seed setting in the material obtained from the crosses (wheat × rye) × *Triticale* all pointed in the same direction. Additional evidence of the same kind was obtained from the crosses *Triticum vulgare* × *Triticale*.

The barrier between hexaploidy and octoploidy does not seem to be very strong but is evidently sufficient to maintain an average constancy. This is illustrated best by the fact that *Triticale* RIMPAU, as already emphasized, has remained constant during almost 50 years of cultivation, in spite of the fact that this strain has a rather irregular meiosis [1]. A certain percentage of aberrants is certainly formed, but these aberrants, as indicated by the pollen examination (Table 6), are rather sterile and probably also less vigorous than the euploid plants. The production of many more or less defective kernels also indicates selective elimination.

Finally, chromosome variation in the crosses (wheat × rye) × *Triticale* remains to be considered. The idea of producing new *Triticale* types by pollinating the F_1 hybrids with the pollen of already existing *Triticale* types was suggested by the present writer (1935 b) and KATTERMANN (1936). This procedure is quite analogous to the production of triple hybrids between *Triticum vulgare, turgidum* and rye (MÜNTZING, 1935 a). In this case the *turgidum* × rye hybrids were pollinated with *vulgare* pollen, the simple principle being to use pollen with the same chromosome number as in the unreduced ovules. As discussed more closely in previous papers (MÜNTZING, 1930, 1933, 1936 b), the viability of the zygotes will generally be best if the gametes uniting have the same or approximately the same chromosome number. Due to quantitative disturbances gametes with different chromosomes often give non-viable embryos and seeds.

As is evident from Table 8, chromosome variation in the cross F_1 × *Triticale* was found to be very great, the chromosome numbers of the 201 daughter plants ranging from 35 to 69. Similar results have been reported by VON BERG and OEHLER (1938). Of 35 daughter plants of wheat × rye F_1 hybrids 20 were found to have 54—58 chromosomes. Most of these plants were the result of direct F_1 × *Triticale* crosses. —

[1] It may be mentioned that the allopolyploid nature of RIMPAU's wheat—rye hybrid was observed independently by LINDSCHAU and OEHLER (1935) and the present writer (1935 a, 1936 a).

The two maxima at 49 and 56 in our own material are surrounded continuously by aneuploid numbers. Theoretically, no numbers higher than 56 should be expected, but the occurrence of plants with 57—60 chromosomes may be accounted for by the functioning of ovules with one or a few chromosomes more than the somatic number. Several similar cases are known (cf. MÜNTZING, 1937, pp. 145—147).

In the present case some of the plants in question might also result from the functioning of *Triticale* pollen grains with more than 28 chromosomes. However, the cross $F_1 \times$ *Triticum vulgare* is also known to give some plants with higher numbers than expected (KATTERMANN, 1934, 1935). Since the formation and functioning of *vulgare* pollen grains with more than 21 chromosomes is highly improbable, the ovules of the F_1 hybrids must be responsible for the results observed. Under such circumstances it is certain that hypertetraploid ovules also function, when *Triticale* is the pollen parent.

The occurrence of a plant with $2n = 69$ is remarkable and the exact mode of origin of this plant is impossible to explain. If the pollen grain, which gave rise to this plant, had 28 or approximately 28 chromosomes, which is most probable, the chromosome number of the functional ovule must have been ± 41. The formation of such a hexaploid ovule may, indeed, be figured out, but the process must have been rather complicated.

In this connection attention should be called once more to the observation that two different wheat varieties after crosses with the same rye variety gave F_1 hybrids which behaved quite differently. In the first combination, Standard $\times$ Stålråg, very little progeny was obtained, and this progeny chiefly consisted of plants with low chromosome number. Evidently the hybrid in question had a rather limited capability of forming unreduced, functional ovules. In hybrids of the other cross, Sol III $\times$ Stålråg, on the contrary, many daughter plants were obtained, demonstrating, by their chromosome numbers, that the mother hybrids had produced a much higher frequency of unreduced ovules than in the other hybrid combination. Thus, the relatively slight genotypical differences between two commercial varieties of wheat are sufficient to give hybrids with rye, behaving quite differently with respect to fertility and the chromosome numbers of the next generation. This emphasizes once more the importance and influence of the genotypical constitution of the original material for the properties of polyploid derivatives.

The results reported in the present paper are in many respects fragmentary, but they are sufficient to give some directions for future

work. The data accumulated are also sufficient to show that the *Triticale* forms are no longer simply a small group of botanical and genetical curiosities but a material constituting a new species or genus and a new cereal of considerable theoretical and practical interest. The increased importance of *Triticale* is due to the fact that it is now possible, in various ways, to produce an unlimited number of new strains and to get these strains constant or relatively constant. When variation is rich, selection has great opportunities. It will, indeed, be interesting to see what the breeding work with this material can achieve in the near future.

Acknowledgement. — The present investigation has been greatly facilitated thanks to the assistance of various persons working at the Cyto-genetic Department of the Swedish Seed Association. Especially I wish to express my sincere gratitude to Fil. Kand. H. NORDENSKIÖLD and Dozent Dr. R. FREISLEBEN, Halle, for valuable cooperation.

VI. SUMMARY.

1) Among six constant *Triticale* strains studied significant differences in vigour, fertility and meiotic regularity were found. These strains are also different in physiological and chemical respects.

2) Interstrain crosses succeed with difficulty, the average seed setting being as low as 5,08 per cent. In some crosses the reciprocal combinations give different results.

3) Interstrain hybrids were found to be vigorous. In one of the crosses, which was studied in detail, the F_1 plants were taller than the average height of the parents, but were more sterile than the parents and showed a higher degree of non-conjunction at meiosis. In F_2 there was a significant decrease in vigour as well as in fertility.

4) New *Triticale* strains were produced a) by crosses between constant strains already existing, b) by pollination of primary wheat $\times$ rye hybrids with *Triticale* pollen and c) by crosses between *Triticale* and *Triticum vulgare.*

5) The primary wheat $\times$ rye hybrids may differ in the frequency of functional unreduced gametes. In one case this was found to be due to genotypical differences betweeen the wheat varieties used.

6) Correlations between chromosome number, vigour and fertility

show that the octoploid *Triticale* strains are separated from the hexaploid condition by a zone of reduced vigour and fertility.

Institute of Genetics of Lund University, July 1939.

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TRANSLOKATION, GENSPALTUNG UND MUTATION BEI PISUM

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SAATZUCHTANSTALT WEIBULLSHOLM, LANDSKRONA

(With a Summary in English)

EINLEITUNG.

SEIT einer Reihe von Jahren sind bei *Pisum* Fälle von Semisterilität bekannt. HAMMARLUND (1923) studierte zwei Kreuzungen, in denen u. a. die Genpaare *A—a* und *Gp—gp* spalteten. In der einen dieser zeigten diese beiden Gene starke Koppelung, in der anderen dagegen wurden sie unabhängig voneinander vererbt. Erst viel später wurde im Zusammenhang mit zytologischen Untersuchungen von HÅKANSSON (HÅKANSSON, 1929; HAMMARLUND und HÅKANSSON, 1930; HÅKANSSON, 1931) erkannt, dass diese Erscheinung auf einen Segmentaustausch zwischen nicht-homologen Chromosomen beruhte.

Bei Kreuzung zwischen einem solchen, translozierten und einem nicht-translozierten Typus kommt es bei der Reduktionsteilung in F_1 zur Bildung eines Ringes, eines sog. Amphibivalents laut HÅKANSSON. Bei der Gametenbildung findet dann eine Neukombination zwischen den Chromosomen statt, die zur Folge hat, dass zweimal etwa 25 % Gameten entstehen, denen je ein Chromosomenstück fehlt und die daher letal sind. Es resultieren demnach etwa 50 % lebensfähige und etwa 50 % letale Gameten. In Übereinstimmung hiermit werden dann etwa 50 % tauber Pollen und etwa 50 % Samenansatz in den Hülsen beobachtet.

Etwa gleichzeitig wurden analoge Beobachtungen von RICHARDSON (1929) und von PELLEW and RICHARDSON SANSOME (1932) in Kreuzungen zwischen einer asiatischen Erbsenlinie, »Thibet 7», und Linien aus Duke of Albany gemacht. Ausser Fällen mit einem Vier-Ring (HÅKANSSONS Amphibivalent) wird auch über einen interessanten Fall mit einem Sechs-Ring berichtet, in dem der Gametenausfall nicht wie erwartet 75 % sondern etwas weniger als 70 % betrug. Diesbezüglich sagen die Verff.: »This may indicate that the possibility of any two segments disjoining is increased when the number of chromosomes in the ring is increased». PELLEW (l. c.) findet in einer Kreuzung auch einen

klaren Zusammenhang zwischen Semisterilität und Spaltung im Genpaar *R*—*r* (runde—runzlige Samen). Es wurden folgende Zahlen erhalten:

75 fert. *RR* : 13 semist. *RR* : 18 fert. *Rr* : 174 semist. *Rr* : 62 fert. *rr* : 7 semist. *rr*.

Theoretisch zu erwarten wären nur: 25 % fertile *RR* : 25 % fertile *rr* : 50 % semisterile *Rr*. Vergleiche diesbezüglich Fig. 1 unten. Die Entstehung der übrigen drei Kombinationen wäre zu erwarten bei abermaligem Segmentaustausch, d. h. in F_1 (oder auch in weiteren Generationen).

E. NILSSON (1933 und 1936) hat über mehrere Fälle, sowohl Nachkommen nach einzelnen Pflanzen wie Kreuzungen, berichtet, in denen Semisterilität von etwa 50 % aufgetreten ist. In einem Fall, Nachkommen einer einzelnen in einer Kreuzung aufgetretenen semisterilen Pflanze (N. I), wurde eine sicher grössere Sterilität als 50 % nachgewiesen. Sie betrug 68,6 % und D/m für das Verhältnis 1 fertil : 1 steril war 7,55, also grosse Sicherheit für die Abweichung anzeigend. In einem der Semisterilitätsfälle (N. IV) wurde sowohl in spaltenden N. IV-Parzellen wie in Kreuzungen mit N. IV ein regelmässiges Auftreten eines stark abweichenden und sehr schwach vitalen Typus beobachtet. Das Auftreten desselben wird mit den für N. IV charakteristischen Chromosomenstörungen in Zusammenhang gebracht. Einen Zusammenhang zwischen Genenspaltung und Semisterilität hat E. NILSSON bisher nicht veröffentlicht.

HÅKANSSON (1932 und 1934), der das semisterile Material von E. NILSSON zytologisch untersucht hat, konnte ausser das Auftreten von Amphibivalenten auch das Vorkommen von zwei Vier-Ringen und Trivalenten, Fünf-Ketten und Sechs-Konfigurationen feststellen.

SEMISTERILITÄT UND GENENSPALTUNG.

Durch den Segmentaustausch zwischen zwei nicht-homologen Chromosomen werden zwei Paare Chromosomen eines neuen Typus erhalten, die andere Koppelungsverhältnisse zeigen sollen. Bei Kreuzung von Linien eines solchen abweichenden Typus mit dem »Normaltypus» soll Semisterilität nach dem in Fig. 1 wiedergegebenen Schema auftreten. (Die Bezeichnung »Normaltypus» ist natürlich höchst willkürlich, denn es ist sehr wohl möglich, dass z. B. in gewissen Gegenden Asiens praktisch genommen alle Erbsen einem anderen Chromosomentypus angehören als den bei uns als normal bezeichneten.)

Fig. 1 zeigt schematisch das Auftreten von Semisterilität nach Kreuzung von zwei Linien, die sich durch einen Segmentaustausch in

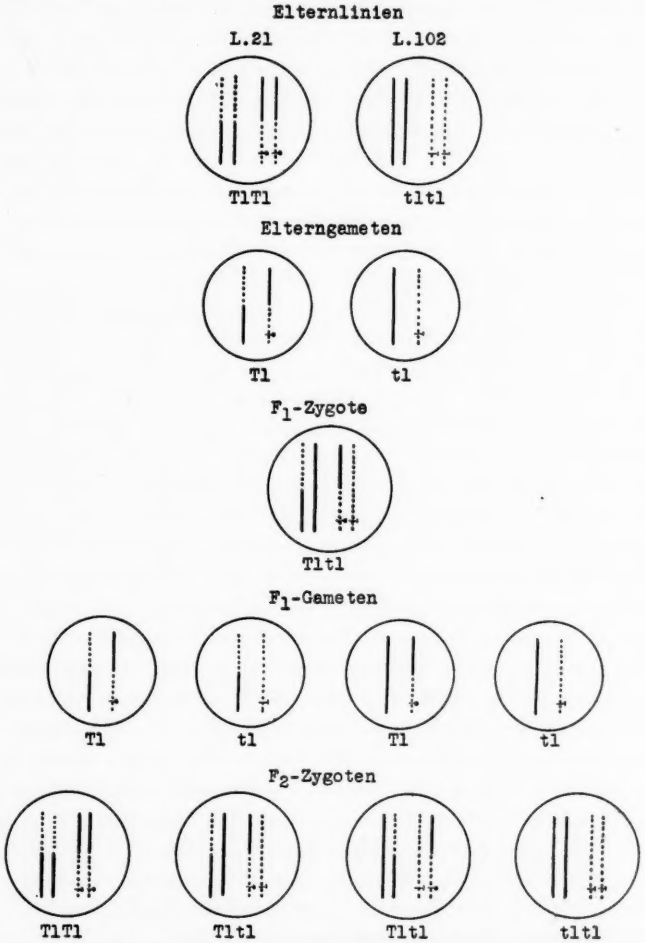

Fig. 1. Schematische Darstellung der Aufspaltung im Genpaar *Tl—tl*, wenn dieses bei sog. Segmentaustausch in einem der translozierten Chromosomen liegt. Wie ersichtlich führen die *Tltl*-Zygoten stets zur Entwicklung von semisterilen Pflanzen.

zwei Chromosomen unterscheiden. Es wurde ein konkretes Beispiel gewählt, nämlich meine Kreuzung Nr. 265, Linie 21 × Linie 102. Welchen von den beiden Eltern wir als »normalen« oder »translozier-

ten» Typus auffassen, ist natürlich vollkommen gleichgültig. Das Schema zeigt uns zwei Erscheinungen. *Erstens*, dass wir in einer solchen Kreuzung *unter den F_1-Gameten 50 %* zu erwarten haben, denen ein Stück eines Chromosoms fehlt und die daher *letal sind*. Das fehlende Chromosomenstück ist das am Austausch beteiligte Segment. *Zweitens*, dass zwischen einem im translozierten Chromosom gelegenen, spaltenden Gen und der Semisterilität in F_2 folgender Zusammenhang bestehen soll. *Die fertilen Pflanzen in F_2 sollen das betreffende Gen nur im homozygot-dominanten bzw. -rezessiven Zustand, die semisterilen sollen es nur im heterozygoten Zustand enthalten.* In Fig. 1 handelte es sich um das bekannte Gen für die Ausbildung von Ranken *Tl*; *tltl*-Pflanzen haben nur Blättchen statt Ranken.

Im folgenden wird eine Reihe von Kreuzungsresultaten mitgeteilt, geeignet um das Problem der Semi- bzw. partieller Sterilität zu beleuchten. Zahlen für den Sterilitätsprozent in F_1 wurden sowohl durch Pollenuntersuchungen wie durch Feststellung des Verhältnisses Samenanlagen: Samen erhalten. Die Pollenuntersuchugen erfolgten an je einer Knospe pro F_1-Pflanze. Zur Feststellung der Weibchensterilität wurden von den F_1-Pflanzen soviele normal entwickelte Hülsen beurteilt bis wenigstens 400 Samenanlagen durchgegangen waren. Gewöhnlich betrug ihre Anzahl 700—900, in einzelnen Fällen bis über 3,000. In einigen Fällen wurde auch für ganze F_2-Generationen je Pflanze die Anzahl Samenanlagen und sich entwickelnder Samen ermittelt. Es sei hervorgehoben, dass der Prozent Samenansatz bei typischer *Semi*-Sterilität, also bei 50 %iger Letalität der Gameten, stets etwas geringer zu erwarten ist als 50 %. Denn auch bei 100 % fertilen Linien findet man in der Regel nicht 100 % Samenansatz sondern etwas weniger. Der Ausfall kann mehrere Prozente erreichen. Ausserdem sei in diesem Zusammenhang erwähnt, dass es Erbsenlinien gibt, die konstant einen gewissen geringeren Prozent steriler Gameten und einen dementsprechenden Ausfall im Samenansatz zeigen (von Dr. E. ÅKERBERG festgestellt, noch unveröffentlicht). Das Auftreten einer solchen genetisch bedingten Sterilität zusammen mit Semisterilität würde den Fertilitätsgrad noch geringer erscheinen lassen.

Kreuzung Nr. 265. — Ausgeführt zwischen L. 21 und L. 102. Linie 21 ist identisch mit H. u. O. TEDINS (1928) 01001, »*Light purple flowered pea*». Sie stammt aus einer Sammlung von H. VON POST, Ultuna Landwirtschaftliche Hochschule, Schweden. Ihre Formel ist, soweit sie hier in Betracht kommt: *A ar b M Pl f R Le Tl.* Linie 102 stammt aus der französischen niedrigen Markerbse *Acacia* (VILMORIN-

ANDRIEUX). Ihre Formel ist: *a Ar B m pl F r le tl.* In Kreuzung 265 war demnach eine Aufspaltung in den neun angeführten Genen zu erwarten.

Die F_1-Generation zeigte eine Pollensterilität, die signifikativ mit 50 % übereinstimmte. Für den Samenansatz resultierte folgendes Verhältnis. 704 Samenanlagen : 350 Samen; die Fertilität, ausgedrückt in entwickelten Samen = 49,7 %. D/m für das Verhältnis fertile : sterile Samenanlagen = 0,15, also ungewöhnlich gute Übereinstimmung ausdrückend.

Die F_2-Generation ergab das Verhältnis 150 fertile : 134 semisterile Pflanzen mit D/m für 1 : 1 = 0,95. Für die oben genannten neun Gene wurden in den beiden Gruppen fertil und semisteril folgende Spaltungen gefunden. Die Werte für D/m sind hier für monohybride Spaltung in jeder dieser Gruppen berechnet.

113 *fert. A* : 37 *fert. a* :	108 *semist. A* :	26 *semist. a*	
D/m =	0,94	1,50	
117 *fert. M* : 33 *fert. m* :	106 *semist. M* :	28 *semist. m*	
D/m =	0,84	1,10	
123 *fert. Le* : 27 *fert. le* :	100 *semist. Le* :	34 *semist. le*	
D/m =	1,98	0,10	
77 *fert. B* : 32 *fert. b* :	87 *semist. B* :	25 *semist. b*	
D/m =	1,05	0,66	
84 *fert. Ar* : 25 *fert. ar* :	94 *semist. Ar* :	18 *semist. ar*	
D/m =	0,50	2,19	
96 *fert. Pl* : 28 *fert. pl.* :	89 *semist. Pl.* :	37 *semist. pl.*	
D/m =	0,62	1,13	
57 *fert. F* : 20 *fert. f* :	67 *semist. F* :	20 *semist. f*	
D/m =	0,20	0,43	
83 *fert. R* : 67 *fert. r* :	134 *semist. R* :	0 *semist. r*	
D/m =	5,57	6,68	
81 *fert. Tl* : 69 *fert. tl* :	134 *semist. Tl* :	0 *semist. tl*	
D/m =	5,95	6,68	

Bei den oben angeführten Spaltungen sind die in *B—b* und *Ar—ar* nur auf die *A*-Individuen (221) berechnet, da *a*-Individuen diesbezüglich nicht unterschieden werden können. Ebenso ist die Spaltung in *Pl—pl* nur auf 250 Individuen, alle ohne *A ar B*, berechnet, da letztere wegen des reduzierten Hilums nicht in *Pl—pl* beurteilt werden können. Schliesslich beziehen sich die Zahlen für die Spaltung in *F—f* nur auf Individuen ohne *A b* (164), da das Vorhandensein von violetten Punkten

auf der Testa bei *A Ar b* und *A ar b* Individuen nicht sicher festgestellt werden kann.

Die mitgeteilten Zahlen zeigen, dass die Spaltung in den sieben Genpaaren *A—a*, *M—m*, *Le—le*, *B—b*, *Ar—ar*, *Pl—pl* und *F—f* in den beiden Gruppen fertil und semisteril befriedigend mit dem monohybriden Verhältnis 3 : 1 übereinstimmt. Ganz anders verhalten sich die beiden Genpaare *R—r* und *Tl—tl*. Für diese wurden in der semisterilen Gruppe überhaupt keine Doppeltrezessiven gefunden, was auch laut dem in Fig. 1 mitgeteilten Schema zu erwarten war. Ein neuerlicher Segmentaustausch in F_1, der das Auftreten von semisterilen *rr*- bzw.

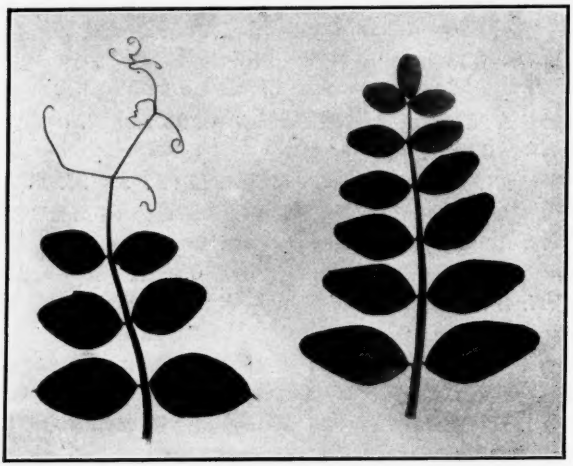

Fig. 2. Links paarig gefiedertes Erbsenblatt mit drahtförmigen, runden Ranken von Linie 21, *Tltl*, rechts unpaarig gefiedertes Blatt der Linie 102, *tltl* (Acacia).

tltl-Pflanzen in F_2 hätte verursachen können, scheint also nicht vorgekommen zu sein.

In diesem Zusammenhang sei hervorgehoben, dass jedoch bei der Beurteilung der F_2-Generation 8 Individuen als semisteril und *rr* bzw. *tltl* klassifiziert worden sind. Alle diese waren indessen sehr schwach entwickelte Pflanzen mit nur ganz wenigen Hülsen und ein Anbau ihrer Samen in F_3 ergab nur fertile Nachkommen, weshalb die in F_2 festgestellte Semisterilität dieser Pflanzen nur eine scheinbare gewesen sein kann. Natürlich ist deshalb das Auftreten solcher Ausnahmen, bedingt durch einen neuerlichen Segmentaustausch, keineswegs ausgeschlossen. Ich wollte jedoch diese Beobachtung besonders anführen, um zu zeigen,

wie vorsichtig man in bezug auf die Sicherheit solcher Klassifikationen sein muss. In anderen Kreuzungen sind jedoch solche Ausnahmen sicher beobachtet worden.

Laut dem Schema in Fig. 1 soll ferner Semisterilität nur im Zusammenhang mit Heterozygotie spaltender und im ausgetauschten Segment gelegener Gene vorkommen. Kreuzung 265 bildet ein ausgezeichnetes Beispiel zur Prüfung dieser Annahme. Die beiden Gene *R* und *Tl* können nämlich in ihrer heterozygoten Form leicht von den Homozygoten

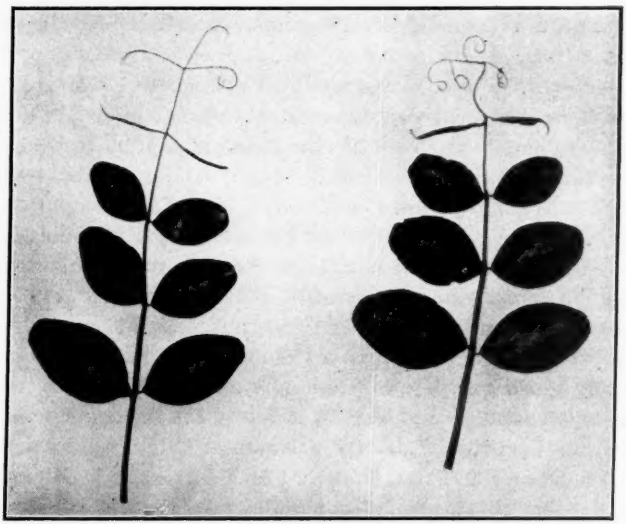

Fig. 3. Blätter der F_1-Pflanzen von Kreuzung Nr. 265, L. 21 × L. 102. Die Ranken sind teils rund, aber einseitig offen, rinnenförmig, teils haben sie die Form von sehr schmalen Blättchen, *Tltl*.

unterschieden werden. Fig. 2 und 3 zeigen die Blätter, den drei Formeln *TlTl*, *Tltl* und *tltl* entsprechend (Erklärung siehe unter den Figuren). Das Genpaar *R*—*r* bezieht sich bekanntlich auf die Samenform rund—runzlig; *Rr*-Pflanzen enthalten in ihren Hülsen demnach beide Formen etwa im Verhältnis 3 *R* : 1 *r*. Eine Analyse des F_2-Materials ergab diesbezüglich folgende Zahlen:

83 *fert. RR* : 0 *fert. Rr* : 67 *fert. rr* : 0 *semist. RR* : 134 *semist. Rr* : 0 *semist. rr*
81 *fert. TlTl* : 0 *fert. Tltl* : 69 *fert. tltl* : 0 *semist. TlTl* : 134 *semist. Tltl* : 0 *semist. tltl*

Diese Zahlen berechtigen zweifellos zur Annahme, dass die *Translokation jenes Pisum-Chromosom betroffen hat, in dem die beiden stark*

gekoppelten Gene R und Tl gelegen sind. Dies stimmt gut mit der Beobachtung von PELLEW (in PELLEW and RICHARDSON SANSOME, 1932), die in ihrer Kreuzung Duke of Albany × Thibetan pea eine ebensolche Spaltung in *R* : *r* beobachtet haben. Sie fanden überdies ca. 8 % Pflanzen in den obigen 0-Gruppen, also semisterile *RR* und *rr* sowie fertile *Rr*-Individuen, die vielleicht auf eine neuerliche Translokation in F_1 zurückgeführt werden könnten. Eine Nachprüfung in der folgenden Generation scheint allerdings nicht stattgefunden zu haben. — Welches das zweite, an der Translokation beteiligte Chromosom ist, darauf kann auf Grund des vorliegenden Materials nicht geschlossen werden.

Kreuzung Nr. 208. — Ausgeführt zwischen L. 58 und L. 151. Linie 58 stammt aus der deutschen Zuckererbsensorte *Graue Posthörnchen.* Diese hat, soweit dies hier in Betracht kommt, folgende genotypische Konstitution: *M F n v pur.* Linie 151 stammt aus der Sorte *Purpurviolettschotige.* Ihre Formel ist *m f N V Pur.*

Die F_1-Generation zeigte eine mit 50 % gut übereinstimmende Pollensterilität; 244 normale : 226 taube Körner mit D/m für 1 : 1 = 0,83. Für den Samenansatz ergaben sich folgende Zahlen. 2709 Samenanlagen : 1395 Samen; die Fertilität, ausgedrückt in entwickelten Samen = 51,5 %. D/m für das Verhältnis fertile : sterile Samenanlagen = 1,55, also gute Übereinstimmung anzeigend.

Die F_2-Generation ergab das Verhältnis 254 fertile : 221 semisterile Pflanzen mit D/m für 1 : 1 = 1,51. Sämtliche Zahlen sind demnach für typische *Semi*-Sterilität signifikativ. Für die Gene *M*, *F*, *N* und *V* wurden in den beiden Gruppen fertil und semisteril folgende Spaltungen gefunden. D/m-Berechnung wie früher. Das Genpaar *Pur—pur* wurde nicht berücksichtigt, da in diesem starke Mutabilität von *Pur* in der Richtung nach *pur* auftrat. Es bestehen vier Allele von *Pur*: *Pur* — pur_a — pur_b — *pur.* (Näheres hierüber folgt in einer besonderen Arbeit.)

177 *fert. M* : 77 *fert. m* : 157 *semist. M* : 64 *semist. m*			
D/m =	1,96		1,36
195 *fert. F* : 59 *fert. f* : 174 *semist. F* : 47 *semist. f*			
D/m =	0,65		1,28
186 *fert. N* : 68 *fert. n* : 165 *semist. N* : 56 *semist. n*			
D/m =	0,65		0,12
138 *fert. V* : 116 *fert. v* : 220 *semist. V* : 1 *semist. v*			
D/m =	7,61		8,44

Die gefundenen Zahlen zeigen, dass die Spaltung in den drei Genpaaren *M—m*, *F—f* und *N—n* in den beiden Gruppen fertil und semisteril gut mit dem Verhältnis 3 : 1 übereinstimmt. Stark abweichend verhält sich in dieser Kreuzung das Genpaar *V—v*. In der semisterilen Gruppe wurde nur eine *vv*-Pflanze verzeichnet und in bezug auf diese ist noch nicht sicher ob sie wirklich hierher gehört. Für diese gilt das diesbezüglich schon in Kreuzung Nr. 265 Gesagte.

Die für das Genpaar *V—v* und Semisterilität erhaltenen Zahlen stimmen gut mit dem laut Schema in Fig. 1 zu erwartenden Verhältnis 1 fertil *V* : 1 fertil *v* : 2 semisteril *V* : 0 semisteril *v*. Eine Aufteilung in Heterozygote und Homozygote ist in bezug auf *V* undurchführbar. Zweifellos haben wir es aber in *Kreuzung Nr. 208* mit dem *gleichen Typus von Semisterilität* zu tun wie in der vorigen Kreuzung, Nr. 265, nur dass *jetzt die Translokation ein anderes Pisum-Chromosom getroffen hat, nämlich das die Gene V und Le enthaltende.*

Kreuzung Nr. 78. — Ausgeführt zwischen L. 21 und L. 110. Linie 21 ist bereits früher beschrieben; Formel *A ar b M Pl N V*. Linie 110 stammt aus der schwedischen Brecherbse Kungsärt (= Königserbse). Ihre genotypische Konstitution ist: *a Ar B m pl n v*. Es ist in Kreuzung 78 also Spaltung in diesen sieben Genen zu erwarten.

Der Pollen der F_1-Generation war typisch 50 % steril; gezählt 624 normale : 625 taube Körner. Für den Samenansatz in F_1 ergab sich: 2988 Samenanlagen : 1433 Samen. Fertilität, ausgedrückt in entwickelten Samen = 48,0 %. D/m für das Verhältnis fertile : sterile Samenanlagen = 2,23.

Die F_2-Generation ergab das Verhältnis 212 fertile : 235 semisterile Pflanzen mit D/m für 1 : 1 = 1,09. Es besteht demnach sowohl in F_1 wie in F_2 gute Übereinstimmung mit bei *Semi*-Sterilität erwarteten Zahlen. Für die angeführten sieben Gene wurden folgende Spaltungen beobachtet:

151 *fert. A*	: 61 *fert. a*	: 179 *semist. A*	: 56 *semist. a*	
D/m =	1,27		0,41	
117 *fert. Ar*	: 34 *fert. ar*	: 139 *semist. Ar*	: 40 *semist. ar*	
D/m =	0,66		0,82	
116 *fert. B*	: 35 *fert. b*	: 125 *semist. B*	: 54 *semist. b*	
D/m =	0,47		1,59	
155 *fert. M*	: 57 *fert. m*	: 186 *semist. M*	: 49 *semist. m*	
D/m =	0,63		1,47	

165 *fert. Pl* : 47 *fert. pl* : 106 *semist. Pl* : 49 *semist. pl*
D/m = 0,95 1,47
161 *fert. N* : 51 *fert. n* : 185 *semist. N* : 50 *semist. n*
D/m = 0,32 1,32
153 *fert. V* : 59 *fert. v* : 194 *semist. V* : 41 *semist. v*
D/m = 0,45 2,67

In allen spaltenden Genpaaren wurde also sowohl in der fertilen wie in der semisterilen Individuengruppe monohybride Spaltung gefunden. In dieser Kreuzung, die auch Spaltung im Genpaar *V—v* zeigte, konnte demnach kein Zusammenhang zwischen diesem und der Semisterilität festgestellt werden, was in der vorigen Kreuzung, Nr. 208, der Fall gewesen ist. Es ist natürlich möglich, dass es sich nicht um einen dritten Fall sondern um den schon in Kreuzung Nr. 265 konstatierten handelt, in dem das *R Tl*-Chromosom an der Translokation beteiligt war; diese Gene haben aber in der vorliegenden Kreuzung, Nr. 78, nicht gespaltet.

Kreuzung Nr. 240. — Ausgeführt zwischen L. 21 und L. 206. Betreffs L. 21 siehe Kr. 265. L. 206 ist eine niedrige Brechmarkerbse, die aus Kreuzung Nr. 126 (Witham Wonder × Roi des Gourmands) stammt. Sie hat die Formel: *a Ar B m pl r v n le*, L. 21: *A ar b M Pl R V N Le.*

Der Pollen der F_1-Generation war typisch semisteril: 363 normale : 385 taube Körner. Für den Samenansatz in F_1 resultierte: 2422 Samenanlagen : 1183 Samen. Fertilität, ausgedrückt in entwickelten Samen = 48,9 %. D/m für das Verhältnis fertile : sterile Samenanlagen = 1,14.

Die F_2-Generation ergab das Verhältnis 215 fertile : 216 semisterile Pflanzen mit D/m für 1 : 1 = 0,05. Die Kreuzung ist demnach in jeder Hinsicht typisch semisteril. Von den neun spaltenden Genen zeigten alle mit Ausnahme von *R* sowohl in der fertilen wie in der semisterilen Gruppe monohybride Spaltung. Für das Genpaar *R—r* wurde erhalten:

119 *fert. RR* : 98 *fert. rr* : 213 *semist. Rr* : 1 *semist. rr*
D/m = 6,86 8,28

Wie ersichtlich zeigt die Spaltung in *R—r* sehr gute Übereinstimmung mit der laut Schema in Fig. 1 bei Semisterilität zu erwartenden. Ausserdem wurde 1 sicheres semisteriles *rr*-Individuum angetroffen, was durch Anbau der Nachkommen in F_3 hat bestätigt werden können. In diesem Fall dürfte es daher in F_1 einmal zu einer Translokation mit dem *R*-Chromosom gekommen sein, die die Semisterilität in F_2 aufgehoben hat.

Die vorstehend mitgeteilten vier Kreuzungen, Nr. 265, 208, 78 und 240 dürften zur Beleuchtung der Genenspaltung bei typischer *Semi*-Sterilität genügen. Ausserdem wurden noch 23 andere untersucht, die ebensolche Semisterilität zeigten, und von denen einige nochmals den gleichen Zusammenhang mit den beiden Genpaaren *R—r* und *V—v* aufwiesen. In einer Kreuzung bestand auch ein Zusammenhang zwischen Semisterilität und Spaltung im Gen *Le*, das mit *V* ziemlich stark gekoppelt ist.

Bisher ist demnach genetisch festgestellt, dass *am Segmentaustausch zwischen nicht-homologen Chromosomen bei Pisum jene zwei Chromosomen beteiligt sind, die die beiden Koppelungsgruppen R—Tl bzw. Le—V enthalten.*

PARTIELLE STERILITÄT UND GENENSPALTUNG.

Wie schon in der Einleitung erwähnt wurde, haben PELLEW in einem Fall einen Gametenausfall von etwas weniger als 70 % und E. NILSSON in seinem N. I-Material einen solchen von 68,6 beobachtet. Dass bei *Pisum* verschiedene Grade von Sterilität zu erwarten sind, geht schon daraus hervor, dass bei dieser Pflanze im Zusammenhang mit Translokationen zwischen den Chromosomen von RICHARDSON SANSOME (1932) und HÅKANSSON (1932 und 1934) ausser Vier-Ringen auch Trivalente, Fünf-Ketten und Sechs-Konfigurationen festgestellt worden sind.

Bevor ich an die Besprechung spezieller Fälle schreite, gebe ich eine Übersicht über von mir bisher festgestellte verschiedene Grade von Sterilität in F_1-Generationen. Von den 27 Kreuzungen mit 50 % Sterilität wird hierbei natürlich abgesehen.

Kreuzung Nr.	*Eltern*	*Sterilitätsgrad in F_1* (Die erste Zahl in Klammern bedeutet guter Pollen bzw. erhaltene Samen, die zweite tauber Pollen bzw. nicht entwickelte Samen)
233	L. 58 × L. 234	Etwa 50 % Ausfall an Samen Anscheinend normaler Pollen
311	L. 11 × L. 58	67,9 % Ausfall an Samen (135 : 286) 69,6 % Pollensterilität (653 : 1495)
328	L. 21 × L. 58	68,3 % Ausfall an Samen (713 : 1539) 68,7 % Pollensterilität (936 : 2044)
20	L. 5 × L. 118	32,0 % Ausfall an Samen (397 : 187)

25	L. 5 × L. 102	33,9 %	Ausfall an Samen (284 : 156) Anscheinend normaler Pollen
36	L. 6 × L. 118	37,5 %	Ausfall an Samen (3385 : 2041)
364	L. 257 × L. 457	32,9 %	Ausfall an Samen (1128 : 555)
22	L. 118 × L. 199 a)	32,5 %	Ausfall an Samen (798 : 385)
	b)	62,9 %	Ausfall an Samen (163 : 277)

Da es bei Mitteilung von Prozentzahlen, wie die obigen, für die Beurteilung des Wertes derselben notwendig erscheint ihre Sicherheit einigermassen zu kennen, teile ich als Beispiel die für Kreuzung Nr. 22 erhaltenen tatsächlichen Zahlen in Tabelle 1 mit.

TABELLE 1. *Gefundene Zahlen für Sterilität von Samenanlagen in Kreuzung Nr. 22.*

Gruppe a) 32,5 % Ausfall an Samen			Gruppe b) 62,9 % Ausfall an Samen		
Samenanlagen pro Pflanze	Samen pro Pflanze	% sterile Anlagen	Samenanlagen pro Pflanze	Samen pro Pflanze	% sterile Anlagen
203	139	31,5	140	52	62,9
108	78	27,8	72	35	51,4
134	82	38,8	26	7	73,1
101	77	25,9	57	22	61,4
122	74	33,9	145	47	67,6
94	64	31,9	—	—	—
108	72	33,3	—	—	—
152	106	30,2	—	—	—
38	24	36,8	—	—	—
109	74	32,1	—	—	—
14	9	35,7	—	—	—
1183	798	32,5	440	163	62,9

Ein Kommentar zu den in Tab. 1 angeführten Zahlen dürfte überflüssig erscheinen; es ist ohne weiteres ersichtlich, dass die beiden Serien signifikativ verschieden sind. Und gleich gute Übereinstimmung zwischen den Werten für die einzelnen Pflanzen wurde auch in allen anderen Fällen angetroffen. Für einzelne Kreuzungen mit etwa 50 % Sterilität wurden Werte bis herab zu etwa 43 % Samenansatz gefunden (bei geringerer Individuenanzahl). Ich sehe aber auch in diesen Fällen von einer Berechnung statistischer Werte ab. Diese könnten solchenfalls

leicht einen signifikativen Unterschied zwischen zwei Fällen angeben, und damit nur etwas Scheinbares anzeigen, solange nicht besondere Untersuchungen dargetan haben, dass das gefundene Plus an Samenausfall nicht auf ein modifikatives solches zurückzuführen ist. Denn wie bereits erwähnt, kann der »modifikative» Samenausfall auch bei 100 % fertilen Linien bis zu mehrere Prozente erreichen.

In der obigen Zusammenstellung über den Sterilitätsgrad verteilen sich die Werte, abgesehen von Kr. 233, auf zwei Gruppen. Eine Gruppe mit etwa $^2/_3$ Samenausfall, die Kreuzungen 311, 328 und 22 b, eine zweite mit ungefähr $^1/_3$ Samenausfall, die Kreuzungen 20, 22 a, 25 und 36. In einigen Kreuzungen, sowohl mit etwa 50 % wie mit ca. 33 % Samenausfall, wurde anscheinend nur normaler Pollen beobachtet. Natürlich können Pollenkörner trotz normalen Aussehens nicht funktionsfähig sein, oder auch sie können tauglich sein, aber den mit ihnen gebildeten Zygoten kann die Entwicklungsmöglichkeit fehlen. Ferner sind Fälle beobachtet worden, bei denen in F_1 eine Pollensterilität von 8,5 bis 10,5 % aufgetreten ist. Ob es sich in diesen um eine zufolge Verschiedenheiten in der Chromosomenstruktur der Eltern oder um eine genisch bedingte Sterilität handelt, kann noch nicht gesagt werden. Schliesslich sei erwähnt, dass in mehreren Kreuzungen einzelne Pflanzen mit etwa 50 % Samenausfall angetroffen worden sind. Diese könnten also auf vereinzelt spontan aufgetretenen Austausch zwischen nicht-homologen Chromosomen zurückgeführt werden. Im folgenden werden einige spezielle Fälle behandelt.

Kreuzung Nr. 233. — Ausgeführt zwischen L. 58 und L. 234. Linie 58 siehe Kr. 208. Linie 234 stammt aus einer im Tausch erhaltenen Samenprobe und hat u. a. stark violett gestreifte Samenschale (*Ast*). Ihre Formel ist *m Pl Ast d N td V*. L. 58 hat diese 7 Gene in der anderen Allelenform.

Der Pollen der untersuchten F_1-Pflanzen zeigte normales Aussehen. Für den Samenansatz der F_1 ergab sich 6632 Samenanlagen : 3269 Samen; die Fertilität, ausgedrückt in entwickelten Samen = 49,2 %. D/m für das Verhältnis fertile : sterile Samenanlagen = 1,15.

Die F_2-Generation zeigte das Verhältnis 262 fertile : 218 semisterile Pflanzen mit D/m für 1 : 1 = 2,01, also noch befriedigende Übereinstimmung anzeigend. Alle Werte deuten also — trotz des normalen Aussehens des Pollens in F_1 — auf typische *Semi*-Sterilität. Im Zusammenhang mit dieser wurden folgende Genspaltungen beobachtet:

197 *fert. M* : 65 *fert. M* : 172 *semist. M* : 46 *semist. m*
D/m = 0,07 1,33
202 *fert. Pl* : 60 *fert. pl* : 162 *semist. Pl* : 55 *semist. pl*
D/m = 0,78 0,08
179 *fert. Ast* : 83 *fert. ast* : 174 *semist. Ast* : 44 *semist. ast*
D/m = 2,49 1,64
189 *fert. D* : 73 *fert. d* : 170 *semist. D* : 48 *semist. d*
D/m = 1,07 1,02
204 *fert. N* : 58 *fert. n* : 150 *semist. N* : 68 *semist. n*
D/m = 1,07 2,11
182 *fert. Td* : 80 *fert. td* : 170 *semist. Td* : 48 *semist. td*
D/m = 2,07 1,02
196 *fert. V* : 66 *fert. v* : 218 *semist. V* : 0 *semist. v*
D/m = 0,07 8,53

Für die ersten sechs Genpaare finden wir sowohl in der fertilen wie in der semisterilen Gruppe monohybride Spaltung. Für diese besteht also kein Zusammenhang mit der Semisterilität. Ein ganz unerwartetes Verhalten zeigt hier das Genpaar *V—v*. Innerhalb der fertilen Pflanzengruppe finden wir eine Spaltung, die sehr gut mit Monohybridie übereinstimmt (D/m für 3 : 1 = 0,07), anstatt wie in den im vorigen Abschnitt besprochenen Fällen von Semisterilität das Verhältnis 1 : 1 zu zeigen. In der semisterilen Gruppe erschienen ausschliesslich *V*-Individuen, wie auf Grund des Schemas in Fig. 1 zu erwarten wäre. Für die ganze F_2-Generation wird im Genpaar *V—v* eine Spaltung von 414 *V* : 66 *v* erhalten, also annähernd 7 : 1 bzw. 8 : 1; D/m ist hierfür 0,36 bzw. 0,83.

Für die Erklärung dieser Erscheinung mache ich einstweilen *folgende Annahme*. Die Gameten der F_1-Generation, ihrem Aussehen nach normal, sind voll funktionstauglich. *Der eine der vier Gametentypen, b* in Fig. 4, *gibt ausschliesslich nicht entwicklungsfähige Zygoten und dasselbe gilt auch für die Zygote c* $\times$ *c, der im einen Chromosomenpaar dasselbe Stück zweimal, also ganz fehlt.* Diese Zygoten sind in Fig. 4 durch ein $\times$ gekennzeichnet. Laut dem Schema in genannter Figur führt diese Annahme zu zweierlei:

1) *Semisterile Früchte, etwa 50 % der Samenanlagen wachsen nicht zu Samen aus;*

2) *Im Genpaar V—v erfolgt eine Spaltung im Verhältnis 7 V* : 1 *v.* Die gemachte Annahme, die einstweilen natürlich ganz hypothetischen Charakter hat, erklärt also in ausgezeichneter Weise die gefundenen Resultate. Die Fertilität betrug ja 49,2 % und für das Spaltungs-

verhältnis 7 *V* : 1 *v* betrug D/m nur 0,36. Erwähnt sei schliesslich, dass mir noch zwei weitere Kreuzungen bekannt sind, die ein nahe mit diesem übereinstimmendes Spaltungsverhältnis in *V* : *v* zeigen und die gleichfalls partiell steril sind.

Kreuzungen mit Linie 21. — In der Zusammenstellung über Kreuzungen mit verschiedenem Grade von Sterilität wurden drei angeführt,

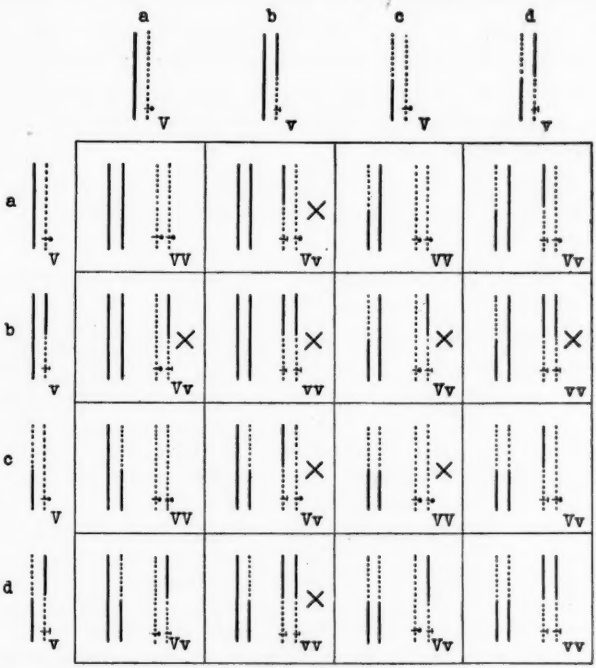

Fig. 4. Schematische Darstellung der Zygotenbildung in Kreuzung Nr. 233. Sämtliche durch *b*-Gameten gebildete sowie die *c*×*c*-Zygote, der ein Stück des einen Chromosoms zweimal fehlt, sind nicht entwicklungsfähig. Es resultiert Semisterilität sowie Spaltung nach 7 *V* : 1 *v*.

Nr. 311, 328 und 22 b, die einen Ausfall an Samen bzw. eine Pollensterilität von etwa $^2/_3$ zeigten. Die erhaltenen Zahlen lagen zwischen 62,9 und 69,9 %. Die eine dieser Kreuzungen, Nr. 328, wurde ausgeführt zwischen L. 21 und L. 58. Beide diese Linien sind bereits früher, in Kreuzung Nr. 265 und 208 im vorigen Abschnitt beschrieben.

In diesen zwei Kreuzungen hat typische *Semi*-Sterilität sowohl hinsichtlich Pollen wie Samenentwicklung festgestellt werden können.

Ferner konnte genetisch nachgewiesen werden, dass die Semisterilität in diesen zwei Kreuzungen durch Translokation in verschiedenen Chromosomen bedingt war. Im einen Fall, Kr. 265, handelte es sich um das die Koppelungsgruppe *R—Tl* enthaltende, im anderen, Kr. 208, um das die Koppelungsgruppe *V—Le* enthaltende Chromosom. Mit welchem Chromosom diese beiden den Segmentaustausch vollzogen haben, ist jedoch aus den Untersuchungen nicht hervorgegangen. Zwei Fälle sind hier denkbar. 1) Der Austausch kann mit zwei verschiedenen Chromosomen stattgefunden haben, oder 2) der Austausch kann mit ein und demselben dritten Chromosom vollzogen worden sein. Im letzteren Fall sollte es von diesem dritten Chromosom drei Typen mit verschiedener Struktur geben.

Linie 21 ist nun in einer ganzen Reihe von Kreuzungen studiert worden, von denen unten die wichtigsten mitgeteilt werden.

Kreuzung	78.	L. 21 × L. 110.	Im vorigen Abschnitt beschrieben. Ca. *50 % steril.*
»	216.	» × L. 208.	Linie 208 stammt aus einer Kreuzung zwischen der dänischen Markerbse *Hamlet* und der englischen *Witham Wonder*. Formel: *a Ar B m pl r i Tl V*, heterozygot im Genpaar *Brev—brev.* Ca. *50 % steril.*
»	240.	» × L. 206.	Im vorigen Abschnitt beschrieben. Ca. *50 % steril.*
»	242.	» × L. 231.	Eine Geschwisterlinie zur eben beschriebenen L. 208. Hat aber *I* in ihrer Konstitution. Ca. *50 % steril.*
»	249.	» × L. 96.	Linie 96 stammt aus *Pois Sabre* von VILMORIN-ANDRIEUX, Paris. Sie ist besonders charakterisiert durch die an der Bauchnaht konkaven Hülsen, *concon.* (Siehe LAMPRECHT, 1936.) Ca. *50 % steril.*
»	259.	» × L. 6.	Linie 6 hat sog. »Umbellatum-Typus»; sie ist rezessiv im Gen *fa.* Formel: *A Ar b M Z Oh R I V fa.* Ca. *50 % steril.*
»	264.	» × L. 232.	Linie 232 stammt von DE WINTON. Formel: *A Ar b m F Z Oh pl s k wb st;* sie hat also u. a. reduzierte Stipeln und ebensolche, schiffchenähnliche Flügel sowie wachsschwache Blätter. Etwa *50 % steril.*

Kreuzung 265. L. 21 × L. 102. Linie 102 stammt aus der bekannten Sorte *Acacia* mit in Blättchen umgewandelte Ranken, *tltl*. Siehe vorigen Abschnitt. *Ca. 50 % steril.*

» 330. » × L. 98. Linie 98 wurde von mir in der schwedischen Sorte *Stens* angetroffen. Sie hat u. a. grünlichgelbe Hülsenfarbe, *oo*. Etwa *50 % steril.*

Sämtliche neun angeführten Kreuzungen zeigten also typische Semisterilität.

Zwischen den zu obigen Kreuzungen verwendeten Linien wurden bisher folgende Kreuzungen ausgeführt und auf Sterilität untersucht: L. 6 × L. 98, L. 6 × L. 110, L. 6 × L. 206, L. 96 × L. 206, L. 102 × L. 206, L. 206 × L. 231. Alle diese Kreuzungen, und überdies noch eine grössere Anzahl Kreuzungen dieser Linien mit anderen waren voll fertil. Wir können daraus schliessen, dass es Linie 21 ist, die das *R—Tl*-Chromosom mit Translokation enthält (siehe i. ü. vorigen Abschnitt, Kr. 265).

Kreuzungen mit Linie 11. — Diese Linie stammt von Dr. H. TEDIN und wurde unter Angabe folgender Formel erhalten: *a ar b M Z pl R I.* Folgende Kreuzungen mit dieser Linie seien hier erwähnt.

Kreuzung 44. L. 11 × L. 112. Linie 112 stammt aus der Ackererbse *Solo* von Svalöf. Die Kreuzung zeigte ca. *50 % Sterilität.*

» 235. » × L. 16. Linie 16 ist identisch mit »KAJANUS' Mutante» aus H. u. O. TEDINS (1928) Material. Sie hat die Formel *A Ar B m Pl F z oh D Fl R I.* Die Kreuzung zeigte ca. *50 % Sterilität.*

» 312. » × L. 98. Siehe die Angaben unter Kr. 330 mit L. 21. Diese Kreuzung zeigte gleichfalls ca. *50 % Sterilität.*

Die drei Linien Nr. 16, 98 und 112 zeigten in sechs bisher untersuchten Kreuzungen mit anderen Linien volle Fertilität; sie scheinen demnach dem »Normaltypus» anzugehören. Da eine Kreuzung zwischen L. 11 und L. 21 (Nr. 310) gleichfalls volle Fertilität zeigte, kann mit Sicherheit geschlossen werden, dass auch Linie 11 den gleichen translozierten Chromosomentypus aufweist wie L. 21, der wie bereits erwähnt, das *R—Tl*-Chromosom betrifft.

Kreuzungen mit Linie 58. — Diese aus »*Graue Posthörnchen*» stammende Linie hat die Formel *A Ar B M F Z Oh pl R I v n.* Vorhin

wurde eine Kreuzung, Nr. 208, besprochen, die typische *Semi*-Sterilität zeigte und die als einen Elter L. 58 hatte. In dieser Kreuzung wurde der charakteristische Zusammenhang zwischen Genenspaltung (*V—v*) und dem Auftreten für Semisterilität festgestellt. Es seien nun einige Kreuzungen angeführt, die darlegen, dass es L. 58 ist, die den translozierten Typus hinsichtlich des *V—Le*-Chromosoms darstellt.

Kreuzung	4.	L. 58 × L. 102.	Linie 102 stammt aus der Sorte *Acacia, tl r.* In vorliegender Kreuzung wurde ca. *50 % Sterilität* beobachtet, aber kein Zusammenhang mit den beiden Genen *R* und *Tl*, die spalteten, dagegen war dies hinsichtlich *V* und *Le* der Fall. Da L. 102 laut früherem dem Normaltypus angehört, sollte also L. 58 einem im *V—Le*-Chromosom translozierten Typus angehören.
»	90.	» × L. 117.	Linie 117 stammt aus der englischen Markerbsensorte *Onward.* Formel: *a Ar B m pl r i.* Die Kreuzung zeigte ca. *50 % Sterilität.*
»	92.	» × L. 177.	Linie 177 stammt aus der englischen Sorte *Autocrate.* Formel: *a Ar B m pl r i.* Die Kreuzung zeigte ca. *50 % Sterilität.*
»	208.	» × L. 151.	Wurde im vorigen Abschnitt besprochen. Ca. *50 % Sterilität.*
»	337.	» × L. 98.	L. 98 siehe Angaben unter Kr. 330 mit L. 21. *Sterilität* = ca. *50 %.*

Da die mit L. 58 gekreuzten Linien in Kreuzungen mit mehreren Linien vom Normaltypus keine Sterilität gezeigt haben, erscheint die Annahme berechtigt, dass Linie 58 durch abweichende Chromosomenstruktur ausgezeichnet ist.

Kreuzungen mit Linie 118. — Diese Linie stammt aus der sehr frühen Kneifelerbse *Extra Rapid* von W. Weibull, Landskrona. In bezug auf diese ist bereits durch HAMMARLUNDS (l. c.) und E. NILSSONS (l. c.) Resultate bekannt, dass sie bei Kreuzung mit Linien vom Normaltypus Semisterilität gibt. Ich teile einige meiner Kreuzungen mit, die dies bestätigen.

Kreuzung	18.	L. 118 × L. 1.	Linie 1 stammt aus der deutschen Markerbse *Goldkönig* und ist besonders charakterisiert durch Rezessivi-

tät im Gen *o* (grünlichgelbe Hülsenfarbe). Diese Kreuzung zeigte ca. *50 % Sterilität.*

Kreuzung 19. L. 118 × L. 96. Betr. L. 96 siehe Kr. 249 mit L. 21. Die Kreuzung zeigte ca. *50 % Sterilität.*

» 98. » × L. 175. Linie 175 ist eine aus Norrland unter dem Namen *Råneå* erhaltene Kneifelerbse. Die Kreuzung zeigte ca. *50 % Sterilität.*

» 199. » × L. 206. Betr. L. 206 siehe Kr. 240 im vorigen Abschnitt. Die Kreuzung zeigte ca. *50 % Sterilität.*

Diese Beobachtungen, sowie dass die zur Kreuzung mit L. 118 verwendeten Linien in anderen Kreuzungen mit dem Normaltypus volle Fertilität geben, bestätigen die bisherigen Befunde von HAMMARLUND und E. NILSSON, laut denen *Extra Rapid* einen translozierten Chromosomentypus enthalten soll.

Ein Rückblick auf die vorstehend mitgeteilten Resultate zeigt, dass es *wenigstens zwei genetisch sichergestellte Typen von Chromosomentranslokation bei Pisum gibt. Der eine betrifft das die Koppelungsgruppe R—Tl enthaltende Chromosom*, meine Linien 11 und 21, *der andere dasjenige mit der Koppelungsgruppe V—Le*, meine Linien 58 und 118.

Welche Grade von Sterilität sind nun zu erwarten, wenn zwei solche Typen miteinander gekreuzt werden? Unter der Voraussetzung, dass der Segmentaustausch in beiden Fällen mit je einem anderen zweiten Chromosom stattgefunden hat, wird es bei der Reduktionsteilung in F_1 offenbar zur Bildung von zwei Vier-Ringen kommen, was dann — wenn das Fehlen der betreffenden Chromosomstücke überhaupt funktionsuntaugliche Gameten bzw. nicht entwicklungsfähige Zygoten bedingt — theoretisch zu 75 % Sterilität führen soll. Verursacht nur das Fehlen des einen Chromosomenstücks untaugliche Gameten bzw. nicht entwicklungsfähige Zygoten, so resultiert auch in einem solchen Fall 50 % Sterilität.

Ganz anders liegen die Verhältnisse, wenn der Segmentaustausch der beiden Chromosomen — in meinen eben genannten Fällen also des *R—Tl*- und des *V—Le*-Chromosoms — mit einem gemeinsamen dritten Chromosom stattgefunden hat. Solchenfalls resultiert in F_1 ein Sechs-Ring, der dann, wie in Fig. 5 schematisch dargestellt ist, zur Entstehung von acht verschiedenen Gameten führt. Je nachdem ob das

Fehlen irgend eines Segments, oder nur von bestimmten oder eines gewissen der drei zu Gameten- bzw. Zygotensterilität führt, werden dann ganz verschiedene Grade von Sterilität zu beobachten sein.

Wir wollen einige dieser Fälle in Betracht ziehen. In Fig. 5 sollen darstellen: das vollausgezogene Chromosom = das *R—Tl* enthaltende,

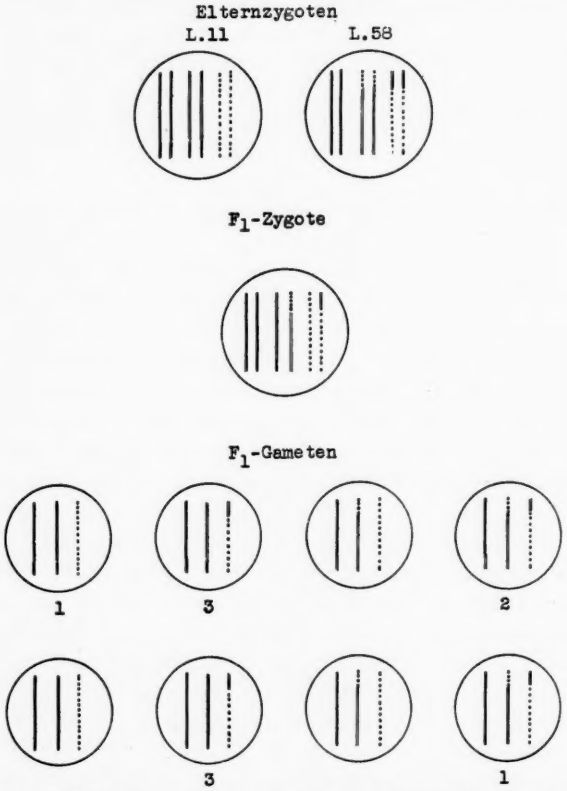

Fig. 5. F_1-Gametenbildung nach Kreuzung von zwei Linien, die verschiedene Translokationstypen repräsentieren. Näheres siehe Text.

das punktierte = das *V—Le* enthaltende und das gestreifte = das noch unbekannte, dritte Chromosom, mit dem die beiden vorigen transloziert vorkommen. Linie 11 und 21 stellen danach Typen dar, bei denen ein Austausch eines Segments zwischen dem *R—Tl*- und diesem unbekannten Chromosom stattgefunden hat, und L. 58 bzw. 118 Typen, bei denen ein Segment des *V—Le*-Chromosoms mit einem anderen Stück

des unbekannten Chromosoms ausgetauscht worden ist. Fig. 5 zeigt in der ersten Reihe die beiden Elternzygoten zu Kreuzung Nr. 311, ausgeführt zwischen L. 11 und L. 58. Darauf folgt die entsprechende F_1-Zygote. Unter der Voraussetzung, dass in F_1 kein neuerlicher Austausch, kein neuerliches Crossingover zwischen den beteiligten sechs Chromatiden stattfindet, sind die in Fig. 5 eingezeichneten 8 verschiedenen F_1-Gameten zu erwarten.

In bezug auf die Sterilität dieser F_1-Gameten dürften folgende Fälle in Betracht zu ziehen sein:

1) Es sind nur jene Gameten fertil, die alle drei Chromosomen ganz enthalten; dies sind nur zwei der acht in Fig. 5 (mit 1 bezeichnet). Diese Annahme sollte also in 75 %-iger Sterilität resultieren.

2) Auch jene Gameten sind fertil, denen nur ein Stück des *R—Tl*-Chromosoms fehlt. Diese Gametensorte ist in Fig. 5 mit 2 bezeichnet. Hierdurch würde eine Sterilität von 62,5 % resultieren.

3) Ausser den unter 1) genannten Gameten sind noch jene fertil, denen nur ein bestimmtes Stück des *V—Le*-Chromosoms fehlt. Diese sind in Fig. 5 mit 3 bezeichnet. In diesem Fall sollte die Hälfte der Gameten steril sein, was also den Eindruck typischer *Semisterilität* machen würde.

4) Es sind die unter 1), 2) und 3) genannten Gameten fertil. Dies sollte eine partielle Sterilität von 37,5 % verursachen.

Betrachten wir nun die auf Seite 441 angeführten Fälle von Kreuzungen mit partieller Sterilität, so finden wir, dass in diesen gerade Sterilitätsprozente beobachtet worden sind, die recht gut mit den vorstehend laut Punkt 2) und 4) zu erwartenden übereinstimmen. So ist laut Annahme unter 2) eine Sterilität von 62,5 % zu erwarten. In drei Kreuzungen, Nr. 311, 328 und 22 b, resultierten die Werte 67,9 %, 68,3 % und 62,9 %. Und laut Annahme unter 4) ist eine Sterilität von 37,5 % zu erwarten. In den fünf Kreuzungen Nr. 20, 25, 36, 364 und 22 a resultierten die Werte von 32,0 %, 33,9 %, 37,5 %, 32,9 % und 32,5 %. Damit ist natürlich noch nicht bewiesen, dass die von mir oben gemachten Annahmen wirklich die richtigen sind. Erst bei einer Analyse solcher Kreuzungen, in denen überdies mehrere Gene spalten, die in den an der Translokation beteiligten Chromosomensegmenten gelegen sind, wird es möglich sein bindende Beweise vorzulegen. Einstweilen dürfte diesen Annahmen der Wert einer Arbeitshypothese zukommen.

Die oben unter 1) bis 4) gemachten Annahmen für das Auftreten von verschiedener partieller Sterilität beziehen sich alle auf denselben Typus von Segmentaustausch, nämlich zwischen zwei Chromosomen

mit einem gemeinsamen dritten, sowie dies in Fig. 5 schematisch dargestellt ist. Solche Fälle sind überdies durch das Auftreten eines Sechs-Ringes bei der Reduktionsteilung in F_1 chakterisiert. Welche sind nun die Gründe, dass gerade ein bestimmter der vier Fälle realisiert wird? Als hier entscheidend erachte ich die genotypische Konstitution der Elternlinien. Es wird von den in den fehlenden Chromosomensegmenten liegenden Genen abhängig sein, ob es zur Bildung von funktionsunfähigen oder voll fertilen Gameten kommt. Was den Pollen betrifft, so ist es, wie schon in Kreuzung 233 gezeigt, nicht notwendig, dass funktionsuntauglicher Pollen auch unnormales Aussehen hat. Es kann auch der Fall vorliegen, dass der Pollen keimt, ja dass es zur Bildung von Zygoten kommt, aber dass diese dann nicht entwicklungsfähig sind. Im letzteren Fall, wie in Kr. 233, findet man also nur Samenausfall.

In bezug auf die alternative Verwirklichung der Fälle 1) bis 4) ist besonders Kreuzung 22 a und 22 b von Interesse. Jede dieser Kreuzungen stammt von vier Befruchtungen zwischen den Linien 118 (aus Extra Rapid) und Linie 199 (aus Badenia) her. Die Befruchtungen wurden an verschiedenen Pflanzen ausgeführt; an den Elternpflanzen, die ursprünglich von einem Individuum herstammten, konnten keine Unterschiede beobachtet werden. Und doch wurde in bezug auf partielle Sterilität in diesen beiden Gruppen ein grosser Unterschied festgestellt. Die Zahlen sind in Tab. 1 (S. 442) zusammengestellt. Wie aus dieser ersichtlich, bestand Gruppe a) aus 11 Pflanzen (F_1), die durchschnittlich 32,5 % sterile Anlagen zeigten. Die Werte variierten zwischen 27,8 und 38,8 %. Die Gruppe b), aus 5 Pflanzen bestehend, ergab eine durchschnittliche Sterilität von 62,9 % mit einer Variation von 51,4 bis 73,1 %. Hier besteht natürlich auch die Möglichkeit, dass die Elternpflanzen der einen Gruppe eine abweichende Chromosomenstruktur gehabt haben. Vielleicht können hier schon zytologische Untersuchungen, die jetzt im Gange sind, etwas Klarheit bringen.

Schliesslich ist hier in Betracht zu ziehen, in welchem Grade die partielle Sterilität durch einen Segmentaustausch in F_1 beeinflusst werden kann, wie er durch SANSOME (1932 und 1933) in einem Sechs-Ring hat festgestellt werden können; in diesem speziellen Fall soll der Austausch 78 % erreicht haben. Fig. 6 zeigt schematisch und in Analogie mit Fig. 5 die hierdurch sich ergebenden Gametentypen. Ein Blick auf diese Figur zeigt ohneweiters, dass wir hier ganz die gleichen Alternativen 1) bis 4) erwarten können, wie sie vorhin mit Hinblick auf Fig. 5 besprochen worden sind. Auch hier (Fig. 6) gibt es nur zwei unter den

acht Gameten, die alle drei Chromosomen ganz enthalten und daher fertil sein sollen. Ferner gibt es eine Gamete, der nur ein Stück des *R—Tl*-Chromosoms (das voll ausgezogene) fehlt und ferner zwei, denen nur ein Stück des *V—Le*-Chromosoms fehlt, also genau wie in Fig. 5. Die Struktur im übrigen ist allerdings abweichend, und könnte daher auch darauf einen Einfluss haben, welche der genannten vier Alternativen verwirklicht wird.

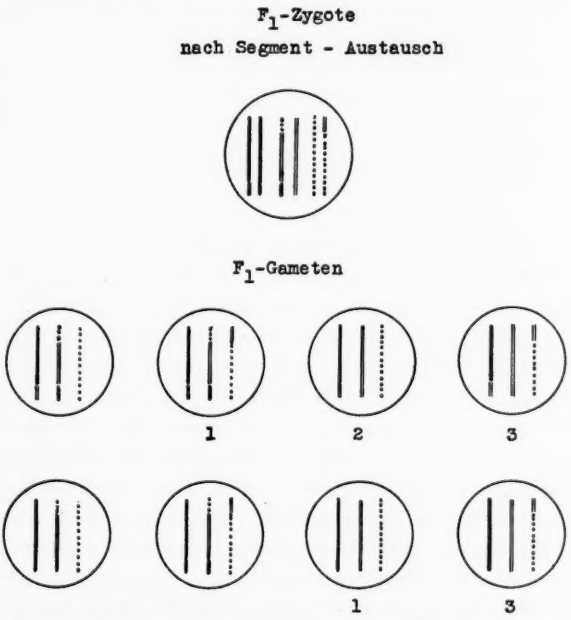

Fig. 6. F_1-Gametenbildung nach Kreuzung der gleichen Linien wie in Fig. 5, jedoch nach bei der Reduktionsteilung in F_1 erfolgtem Segmentaustausch. Näheres siehe Text.

Ausser der binnen der Fehlergrenzen liegenden Variation, werden die Werte für patielle Sterilität noch durch drei weitere Erscheinungen beeinflusst werden können. 1) Durch den modifikativen Ausfall von Zygoten, wie er auch bei voll fertilen Linien mehr oder weniger deutlich auftritt; ein solcher erhöht dann den Sterilitätsprozent. 2) Durch das Auftreten in solchen Kreuzungen von genbedingtem Ausfall an Gameten oder Zygoten; in solchen Fällen bedarf es einer besonderen Analyse in weiteren Generationen um die beiden Arten von Sterilität trennen zu können. 3) Durch das Auftreten eines Segmentaustausches

zwischen nicht-homologen Chromosomen in F_1 solcher Kreuzungen, wodurch ein Teil der sterilen Typen in »Normaltypen« rückumgewandelt werden könnte. Dass einzelne semisterile Pflanzen auftreten können, ist bekannt. Selbst habe ich in mehreren Kreuzungen einzelne Pflanzen, mit Semi- bzw. partieller Sterilität angetroffen. Über die Ursache dieses Auftretens, d. h. der Bildung eines translozierten Typus, ist jedoch so gut wie nichts bekannt.

TRANSLOKATION UND MUTATION.

Linie 58 aus der deutschen dickwandigen Zuckerbrecherbse »*Graue Posthörnchen*« ist vorhin in Kr. 208 erwähnt worden. Ihre Formel ist: *A Ar B M F Z Oh pl R I P v n Le*. Diese Linie wurde von mir seit 1927 gebaut. Wiederholt sind in derselben sog. Kneifelerbsen-Mutationen aufgetreten. Diese beruhen bekanntlich auf der Neigung des Gens *v* zu *V* zu mutieren, welche Erscheinung von mehreren Forschern studiert worden ist (siehe u. a. WELLENSIEK, 1929). Eine solche aus Linie 58 ausgelesene Kneifelerbsen-Pflanze wurde als Linie 59 weitergebaut. Sie konnte von L. 58 nur durch die Ausbildung der starken Membran der Kneifelerbsen, *P V*, unterschieden werden. Im Laufe der Jahre sind mit beiden Linien Kreuzungen ausgeführt worden, die dann auch auf Semi- und partielle Sterilität untersucht wurden. Linie 58 ist diesbezüglich bereits in den beiden vorigen Abschnitten gut charakterisiert worden. Sie gab bei Kreuzung mit dem Normaltypus typische Semisterilität, wobei auch festgestellt werden konnte, dass sie im *V—Le*-Chromosom transloziert ist. Bei Kreuzung mit L. 21, die im *R—Tl*-Chromosom transloziert ist, resultierte eine partielle Sterilität von 69,7 % (Kr. 311). Nun wurde auch eine Kreuzung zwischen L. 59 und L. 21 ausgeführt, Kr. 329, und diese zeigte keineswegs die hohe Sterilität, die mit L. 58 erhalten wurde, sondern 49,8 %.

Wir stehen hier vor der Erscheinung, dass eine Linie, die aus einer anderen durch Mutation in einem einzelnen Gen, $v \rightarrow V$, erhalten worden ist, bei Kreuzung mit einer gemeinsamen dritten Linie (L. 21) einen bestimmt anderen Sterilitätstypus aufweist. Mit Hinblick auf das im vorigen Abschnitt zur Erklärung des Auftretens verschiedener partieller Sterilität in Kreuzungen zwischen Linien desselben Translokationstypus Angenommene, könnte man zuächst vermuten, dass es sich im Zusammenhang mit der Genmutation um einen Fall wie den unter 3) angeführten handelt, bei dem scheinbare Semisterilität auftritt.

Ob diese Annahme richtig ist, darauf wird die Kreuzung zwischen

L. 58 und der aus ihr durch Mutation von $v \rightarrow V$ entstandenen L. 59 Antwort geben. Diese Kreuzung, Nr. 336, war annähernd semisteril; es wurde ein Samenansatz von 43,2 % in F_1 gefunden. Damit kann zweifellos als festgestellt betrachtet werden, dass gleichzeitig mit der Mutation im Genpaar *V—v* auch ein Crossingover zwischen nicht-homologen Chromosomen stattgefunden hat. Höchst wahrscheinlich hat es sich hierbei um das *V—Le*-Chromosom gehandelt; denn nach den bisherigen Kreuzungsresultaten zu urteilen gehört L. 59 dem Normaltypus an.

Von besonderem Interesse wäre hier zu wissen, was als das Primäre aufgefasst werden soll, die Genmutation oder der Austausch zwischen den nicht-homologen Chromosomensegmenten, von denen das eine Träger eben des Gens *V* ist. Selbst neige ich zur Ansicht, dass die Genmutation das Primäre ist. In einem grossen Kreuzungsmaterial habe ich die Beobachtung gemacht (unveröffentlicht), dass stärkere Heterozygotie — namentlich in F_1 von Kreuzungen — eine höhere Mutationsfrequenz auslöst. Die Beobachtungen sprechen auch dafür, dass dies besonders für ein Chromosom gilt, in dem eine grössere Heterozygotie auftritt; gerade in einem solchen soll die Mutationsfrequenz erhöht werden.

Das Auftreten eines Segmentaustausches zwischen nicht-homologen Chromosomen kann kaum als Zufall aufgefasst werden. Die Gene haben wir uns als grössere Moleküle, untergebracht in einer kolloidalen Grundsubstanz, das ganze ein kolloidales System, ein Chromosom, bildend vorzustellen. Die Gene eines Chromosoms müssen, um eben in einem Chromosom vereint sein zu können, sich in ihrer Gemeinschaft in einem Zustand gewisser physikalisch-chemischer Stabilität befinden. Hiermit verbunden ist teils, dass jedes Chromosom gewisse Gene enthält, teils dass jede Art (bzw. Rasse) eine bestimmte Chromosomenzahl aufweist.

Unter physikalisch-chemischer Stabilität ist hier vor allem der elektrostatische Zustand des kolloidalen Systems — repräsentiert durch ein Chromosom — zu verstehen. Die Gesamtheit der Chromosomen hat das ganze Genom, den totalen Genbestand, zu enthalten. Der Genbestand wird auf die einzelnen Chromosomen so verteilt sein, dass jedes dieser ein möglichst stabiles, physikalisch-chemisch im Gleichgewicht befindliches, kolloidales System bilden kann. Veränderungen des Baues von Molekülen, von Genen, wie sie z. B. Mutationen darstellen, bedingen stets geringe Änderungen im elektrischen Potential. Kolloidale Systeme sind gegen solche Änderungen bekanntlich sehr empfindlich. Ist nun die physikalisch-chemische Stabilität eines solchen Systems an

und für sich nicht gross, was gerade in gewissen *Pisum*-Chromosomen der Fall zu sein scheint, so *kann* wahrscheinlich *die Mutation eines gewissen Gens die Stabilität im betroffenen Chromosom schon soweit vermindern, dass dies den Austausch eines Segments mit dem eines nicht-homologen Chromosoms auslösen kann, hierdurch grössere Stabilität herbeiführend.*

Einen solchen Fall sollte meine oben besprochene Mutation im Gen *V* in Linie 58 darstellen. Die Mutation von *v* zu *V*, die Entstehung von Linie 59 aus Linie 58, war gleichzeitig mit einem Segmentaustausch zwischen nicht-homologen Chromosomen verknüpft; das translozierte *V—Le*-Chromosom von L. 58 wurde wahrscheinlich in den Normaltypus zurückverwandelt.

Ferner gebe ich hier der Ansicht Ausdruck, dass *durch Kreuzung in F_1 in einem Chromosom hervorgerufene, stärkere Heterozygotie die Mutationsfrequenz in eben diesem Chromosom erhöhen kann. In solchem Zusammenhang beobachtete Mutationen einzelner Gene deute ich gleichfalls als Reaktionen zufolge einer Störung der physikalisch-chemischen Stabilität im Chromosomenkörper und geeignet diese zu verbessern.*

SUMMARY.

1. The author reports the segregation results of four crossings, showing typical half-sterility. In addition to the normal type, two other types, in which an interchange between non-homologous chromosomes had taken place, could be characterized by genetic analysis.

2. The connection between genic segregation and sterility is expressed by the ratio 1 *fertile A* : 1 *fertile a* : 2 *half-sterile A* (= *Aa*) : 0 *half-sterile a* (cf. Fig. 1). In the two types of interchange genetically analysed the chromosomes concerned were *R—Tl* and *V—Le*.

3. A crossing is recorded, which showed apparently normal pollen, but about 50 per cent. of the ovules did not develop into seeds. The connection between half-sterility and segregation in the gene pair *V—v* was in this case expressed by the ratio 3 *fertile V* : 1 *fertile v* : 3 (or 4) *half-sterile V* : 0 *half-sterile v*. Thus, as a whole this crossing showed a segregation into 7 (or 8) *V* : 1 *v*.

4. In explanation of this phenomenon it is assumed that one of the four types of gametes (*b* in Fig. 4), which carries, among others, the recessive gene *v*, forms exclusively lethal zygotes, giving a segregation of 7 *V* : 1 *v* and a typical half-sterility. The same thing may also apply to the zygote $c \times c$ (v. Fig. 4), which lacks the same chromosome

piece twice, consequently altogether; in that case the result will be the segregation 8 *V* : 1 *v* and nearly half-sterility.

5. Several cases of partial sterility are recorded. The degrees of sterility met with are evenly distributed between two groups, one group having 63—68 per cent. sterility, the other 32—37 per cent. sterility. One cross was found in which the progeny of half the number of fertilizations belonged to one group, while the other half belonged to the other group (v. Table 1).

6. It was ascertained that both these groups of sterility are obtained when two lines, each differing in its way by interchange from the normal type, are crossed. This interchange, however, probably took place with one and the same third chromosome. Thus, the interchange obviously does not give rise to two figures-of-four, which would show about 75 per cent. sterility, but to a figure-of-six. This figure-of-six may, according to what types of gametes that become sterile or produce sterile zygotes, bring about a partial sterility of theoretically 75 per cent., 62,5 per cent., 50 per cent. and 37,5 per cent. (v. Fig. 5). The two values 62,5 and 37,5 per cent. agree very well with the values found.

7. On the assumptions made in § 6, the interchange between the chromatids in figures-of-six, found by SANSOME, gives rise to the same degrees of partial sterility (cf. Fig. 6).

8. One case is recorded in which the mutation from *v* to *V* in a pure line was associated with a simultaneous interchange in the chromosome containing this gene. The cross between the mother line and the offspring of the mutated plant gave half-sterile descendents.

9. With regard to one case, mentioned in § 8, the author is of opinion that the mutation should be regarded as the primary occurrence. The mutation in a gene *might* be able to reduce the stability of the chromosome involved so that an interchange between non-homologous chromosomes takes place, and thus bring about a more stabilized condition.

10. It is also maintained that by means of a stronger heterozygosity in a chromosome, occurring, for instance, in F_1 after crossing, the mutation frequency in that chromosome would be increased. Mutations in solitary genes observed in such a connection are likewise interpreted as reactions due to a disturbance of the physico-chemical stability of the colloidal system of the chromosomal body.

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GENSTUDIEN AN PISUM SATIVUM

IV. ÜBER VERERBUNG VON WACHSLOSIGKEIT UND EIN NEUES GEN FÜR LOKALE AUSBILDUNG VON WACHS, Wsp

VON *HERBERT LAMPRECHT*

SAATZUCHTANSTALT WEIBULLSHOLM, LANDSKRONA

(With a Summary in English)

BISHER BEKANNTE GENE FÜR WACHSLOSIGKEIT.

ÜBER die Vererbung der Wachslosigkeit bei *Pisum* ist bisher folgendes bekannt. VILMORIN (1910, 1913) hat bei Kreuzung zwischen einer wachslosen, *Pois »chenille»*, und einer stark wachsigen Form, *Pois de momie,* wachsige F_1 und in F_2 Spaltung nach 3 wachsig : 1 wachslos gefunden (138 : 39; D/m = 0,91). Eine Kreuzung zwischen zwei wachslosen Formen, *Pois Emereva* und *Pois »chenille»*, gab wachsige F_1 und in F_2 Spaltung im Verhältnis 9 wachsig : 7 wachslos (50 : 41; D/m = 0,25). Eine nähere Charakteristik der Wachslosigkeit dieser Formen, ob vollständig wachslos oder nur wenig wachsig oder in gewissen vegetativen Teilen abweichend, wurde nicht mitgeteilt.

E. v. TSCHERMAK (1912) fand monohybride Spaltung nach 3 wachsig : 1 wachslos (35 : 18; D/m = 1,51).

WHITE (1917) hat auf Grund dieser Ergebnisse zwei Gene für die Ausbildung von Wachslosigkeit aufgestellt, *Bl* und *W*.

MEUNISSIER (1922), der für »Wachslosigkeit» das Symbol *Bl* statt *W* verwendet hat (siehe WELLENSIEK, 1925 c, S. 348—350), findet auch monohybride Spaltung: 42 *W* : 7 *w*; D/m = 1,73.

WELLENSIEK (1925 a, 1925 b) charakterisierte die Wirkung dieser beiden Gene in folgender Weise:

BlBl WW = Viel Wachs,
BlBl ww = Wenig Wachs,
blbl WW } = Kein Wachs.
blbl ww }

Bl sollte als Grundgen für die Ausbildung von Wachs fungieren, und selbst in dominanter Form ohne *W* nur das Auftreten von wenig Wachs bedingen; *blbl*-Pflanzen sollen ganz wachslos sein.

WELLENSIEK (1925 b, 1928 a, 1928 b) hat dann umfangreiche Untersuchungen über die Vererbung der Wachsigkeit angestellt. In bezug auf die Sorten *Emereva, Pois à brochettes* und *Châtenay* konstatiert er, dass sie schwach wachsig sind, wogegen *Johnson's British Empire* ganz wachslos sein soll. WELLENSIEKs (1925 b) Kreuzungen zwischen einerseits Johnson's British Empire und andererseits Pois à brochettes bzw. Châtenay bestätigen die gemachten Annahmen. Diese zwei Kreuzungen zeigten nämlich in F_2 Spaltung im Verhältnis:

9 stark wachsig : 3 schwach wachsig : 4 ohne Wachs.

Insgesamt resultierten folgende Zahlen:

Gefunden:	194	stark	wachsig :	66	schwach	wachsig :	96	ohne Wachs
Erwartet:	200,25	»	» :	66,75	»	» :	89,00	» »
D/m =	0,67	»	» :	0,10	»	» :	0,86	» »

$P > 0{,}05$.

Die weiteren Untersuchungen führten WELLENSIEK (1928 a u. 1928 b) zu der Annahme, dass mehrere Grade von »Wachslosigkeit« unterschieden werden können und dass diese — ausser durch *Bl* — durch die Wirkung von zwei verschiedenen Serien von je drei Allelen bedingt werden. Das Gen *W* wäre danach zu ersetzen durch Wa_2-Wa_1-*wa* und Wb_2-Wb_1-*wb* [WELLENSIEK schreibt $W^a{}_2$ usw.; ich schliesse mich E.NILSSON (1933) an und schreibe nunmehr Wa_2 usw.]. Die von WELLENSIEK zu seinen 28 Kreuzungen verwendeten 8 Sorten wurden in Übereinstimmung hiermit folgendermassen gruppiert:

Haarsteegsche = Express	*Bl* Wa_2 Wb_2	Stark wachsig;
Smaragd · Reuzen, Vlijmsche Krombeck	*Bl* Wa_2 Wb_1	Wachsschwach (zwischen stark und wenig wachsig);
Pois à brochettes, Châtenay ..	*Bl* Wa_2 *wb*	Schwach wachsig;
Emereva	*Bl* Wa_1 Wb_2	Schwach wachsig;
Sutton's Emerald Gem	*Bl wa* Wb_2	Sehr wenig wachsig;
Johnson's British Empire	*bl* Wa_2 Wb_2	Ohne Wachs.

Pflanzen mit dem doppeltrezessiven Grundgen *blbl* sollen demnach stets ganz ohne Wachs sein. Pflanzen mit *Bl* und den verschiedenen Kombinationen von *Wa* und *Wb* sind verschieden stark wachsig.

Spaltungen im monohybriden Verhältnis 3 wachsig : 1 wachslos sind noch von mehreren Forschern gefunden worden. So fand HAMMARLUND (1925) das Verhältnis 368 wachsig : 110 wachslos mit D/m = 1,0. DE HAAN (1927) konstatierte eine solche Spaltung im Ver-

hältnis 462 wachsig : 137 wachslos, mit D/m = 1,20. In diesen beiden Fällen scheint unbekannt gewesen zu sein, um welche Allele sowie ob es sich um *Wa* oder *Wb* gehandelt hat.

SVERDRUP (1927) fand für *Wa* Spaltung nach 792 *Wa* : 243 *wa* mit D/m = 1,13; für *Wb* 2324 *Wb* : 761 *wb* mit D/m = 0,43. Ausgeführte Rückkreuzungen ergaben 1065 *Wb* : 1094 *wb* mit D/m für 1 : 1 = 0,62. Um welche Allelen es sich hier gehandelt hat, scheint gleichfalls unbekannt gewesen zu sein.

LAMPRECHT (1936) findet in drei Kreuzungen folgende Spaltungen im Genpaar Wb_2—*wb*. Kreuzung Nr. 3: 338 Wb_2 : 115 *wb* mit D/m = = 0,19; Kreuzung Nr. 25: 344 Wb_2 : 113 *wb* mit D/m = 0,14; Kreuzung Nr. 68: 349 Wb_2 : 115 *wb* mit D/m = 0,11. Die Symbole Wb_2 und *wb* wurden von mir gewählt, da die »wachslosen» Formen (die Linien Nr. 5 und 7 vom Chenille-Typus) schwach wachsig waren und diesbezüglich genau mit Châtenay, *Bl* Wa_2 *wb* laut WELLENSIEK, übereinstimmten.

E. NILSSON (1933) fand in F_2 seiner Kreuzung Nr. 19, ausgeführt zwischen zwei wachsarmen Linien, Aufspaltung im Verhältnis 597 stark wachsig : 412 wachsarm. D/m für 9 : 7 = 1,87.

Schliesslich hat E. NILSSON (l. c.) ein interessantes *Gen für lokale Ausbildung von Wachslosigkeit* gefunden. In der Erbsensorte English Wonder wurde eine Pflanze entdeckt, bei der die Oberseite der Blättchen ganz frei von Wachs war, während alle übrigen vegetativen Teile derselben stark wachsig waren. Zwei Kreuzungen zwischen dieser Form und der stark wachsigen Muttersorte gaben stark wachsige F_1 und in F_2 monohybride Spaltung im Verhältnis 605 stark wachsig : 201 lokal wachslos mit D/m = 0,03. Weitere Kreuzungen mit anderen stark wachsigen Sorten gaben zusammen 3210 *Wlo* : 1080 *wlo* mit D/m = = 0,27. Das hierfür verantwortliche Genpaar wurde, abgeleitet von Wachs lokalisiert, mit *Wlo*—*wlo* bezeichnet.

ÜBER KOPPELUNGSVERHÄLTNISSE DER GENE FÜR WACHSLOSIGKEIT.

Schon VILMORIN (1910, 1913), der als erster über eine Spaltung in wachsig : wachslos berichtet, konstatiert Koppelung zwischen den beiden Eigenschaften wachslos und »chenille» (d. h. miteinander verklebte reife Samen). Er erhält in drei verschiedenen Kreuzungen folgende Zahlen:

130 *S W* : 6 *S w* : 0 *s W* : 31 *s w*,
57 » : 2 » : 2 » : 21 » ,
265 » : 6 » : 12 » : 57 » .

Für das erste Verhältnis ergibt sich ein Crossingover von 3,50 %, für das zweite ein solches von 5,03 % und für das dritte ein solches von 5,44 %.

MEUNISSIER (1922) fand ein ganz ähnliches Verhältnis, nämlich:

42 *S W* : 2 *S w* : 0 *s W* : 5 *s w*,

wofür ein Crossingover von 4,17 % resultiert.

WELLENSIEK (1925 a und 1929) hat dieselbe Spaltung in fünf verschiedenen Kreuzungen studiert. Seine Untersuchungen zeigen auch, dass es sich um die Allelenserie Wb_2-Wb_1-*wb* handelt, die mit *S* gekoppelt ist. Folgende Verhältnisse wurden gefunden:

1.	100	*S*	Wb_2	: 1	*S wb*	: 2	*s*	Wb_2	: 32	*s wb*;	Crossingover = 2,2 %.
2.	821	»		: 3	»	: 8	»		: 280	» ;	» = 1,0 %.
3.	796	»		: 2	»	: 1	»		: 308	» ;	» = 0,27 %.
4.	822	»		: 43	»	: 9	»		: 198	» ;	» = 5,0 %.
5.	782	»		: 5	»	: 4	»		: 247	» ;	» = 0,9 %.
Sa.=	3586	»		: 66	»	: 30	»		: 1122	» ;	» = 1,89 %.

Die Kreuzungen Nr. 1, 2, 3 und 5 zeigen gute Übereinstimmung, während Nr. 4 deutlich aus der Reihe herausfällt, was in dieser namentlich auf die hohe Anzahl *S wb*-Pflanzen zurückzuführen ist. Vielleicht ist dies auf eine nur teilweise Ausbildung des *ss*-Charakters zurückzuführen (vgl. diesbezüglich LAMPRECHT, 1936, S. 348—358).

DE HAAN (1927) hat eine Kreuzung studiert, in der ausser den beiden Genen für Stammlänge, *La* und *Lb*, auch ein Gen für wenig Wachs gespalten hat. Es zeigte sich, dass das in Frage stehende Gen für Wachsigkeit stark mit einem der beiden Gene *La* und *Lb* gekoppelt sein muss. Es konnte jedoch nicht entschieden werden um welche Gene es sich gehandelt hat.

LAMPRECHT (1936, S. 356—358) veröffentlichte die folgenden bifaktoriellen Spaltungsverhältnisse in *Wb* und *S* aus drei Kreuzungen (Nr. 3, 25 und 68):

Kr. 3.	313	Wb_2S	: 28	Wb_2s	: 87	*wb S*	: 28	*wb s*;	Crossingover = 28,8 %.
Kr. 25.	322	»	: 22	»	: 22	»	: 91	» ;	» = 10,13 %.
Kr. 68.	339	»	: 10	»	: 35	»	: 80	» ;	» = 10,22 %.

L. c. konnte auch gezeigt werden, dass nur in Kreuzung Nr. 25 eine fehlerfreie Beurteilung im Genpaar *S-s* möglich war. Das Verkleben der Samen wird nämlich durch grösseren Abstand zwischen denselben verhindert, wodurch *ss*-Pflanzen als *S* klassifiziert werden können. In Kreuzung Nr. 3 war dies in ziemlich hohem Grade der Fall, was den allzu hohen Crossingover-Prozent von 28,8 bedingt hat. Kreuzung Nr. 25 war frei von dieser Erscheinung und in Kreuzung Nr. 68 ist sie nur relativ schwach aufgetreten. Der richtige Wert für das Crossingover liegt also in diesen drei Kreuzungen bei ungefähr 10 %.

Die beiden Gene *Wb* und *S* zeigen also starke Koppelung, aber die Werte für das Crossingover variieren von etwa 1 bis 10 %. Die bisher vorliegenden Zahlen machen den Eindruck einer Verteilung auf drei Gruppen: Werte um etwa 1—2 %, Werte um ca. 5 % und Werte um ungefähr 10 %. Über die Ursachen dieser Erscheinung kann einstweilen nichts sicheres ausgesagt werden.

Für die Gene *Wb* und *S* ist überdies Koppelung mit einem dritten Gen, *K*, nachgewiesen. PELLEW and SVERDRUP (1923) und SVERDRUP (1927) haben für die Koppelung zwischen *K* und *Wb* folgende Zahlen gefunden:

1. 374 *K* Wb_2 : 164 *K wb* : 174 *k* Wb_2 : 5 *k wb*; Crossingover = 23,8 %.
2. 219 » : 17 » : 19 » : 61 » ; » = 12,02 %.
3. 963 » : 126 » : 104 » : 962 » ; » = 10,68 %.

Kreuzung 1 entspricht dem Typus *Ab* × *aB*, weshalb der Crossingover-Prozent mit einem ziemlich grossen Fehler behaftet sein kann. Nr. 2 entspricht dem Typus *AB* × *ab* und Nr. 3 ist eine Rückkreuzung. Laut diesen Resultaten soll das Crossingover zwischen *K* und *Wb* also bei etwa 10—12 % liegen.

WELLENSIEK (1930) hat als erster eine Kreuzung (Nr. 45) studiert, in der Spaltung in den drei Genpaaren *S—s*, *K—k* und Wb_2—*wb* auftrat. Es wurden folgende Zahlen erhalten:

782 *S* Wb_2 : 5 *S wb* : 4 *s* Wb_2 : 247 *s wb*; Crossingover = 0,9 % (0,8 %).
556 *S K* : 231 *S k* : 247 *s K* : 4 *s k*; Crossingover = 28,1 % (15,7 %).
555 *K* Wb_2:248 *K wb*:231 *k* Wb_2:4 *k wb*; Crossingover = 27,9 % (15,6 %).

Die in den Klammern angegebenen Werte für das Crossingover sind laut WELLENSIEKS Methode (1927) berechnet, die übrigen nach der gebräuchlichen Product Ratio-Methode. In bezug auf die beiden Kombinationen *S—K* und *K*—Wb_2 entsprach die Kreuzung der Form *Ab* × *aB*, weshalb die erhaltenen Werte mit einem ziemlich grossen

Fehler behaftet sein können. So bedingt eine Verminderung der Individuenanzahl in den äusseren beiden Gliedern des Verhältnisses um 20 — was bei einer Individuenanzahl von insgesamt 1038 leicht vorkommen kann — schon eine Verminderung des Crossingover-Wertes von 28 % auf 19,5 %.

Dass die drei Gene *S*, *Wb* und *K* zu einer Koppelungsgruppe gehören, steht auf Grund der vorliegenden Resultate ausser Zweifel. In welcher Reihenfolge sie jedoch liegen, lässt sich an Hand der bisherigen Zahlen nicht sagen. Die beiden Werte für *S*—*K* und *K*—*Wb*, 28,1 % bzw. 27,9 %, sind praktisch genommen gleich. Für die Koppelung *S*—*Wb* fand WELLENSIEK ein sehr geringes Crossingover, 0,9 %.

Selbst habe ich während den letzten Jahren drei Kreuzungen, Nr. 246, 261 und 262, analysiert, in denen gleichzeitige Spaltung in den in Frage stehenden drei Genen *S*, *Wb* und *K* stattgefunden hat. Diese drei Kreuzungen hatten den einen Elter, Linie 232, gemeinsam. L. 232 stammt von DE WINTON und hat folgende Konstitution: *A Ar b m f Z mp s k wb st.* Kreuzung Nr. 246 wurde ausgeführt zwischen L. 232 und L. 19, welch letztere identisch ist mit H. u. O. TEDINS 0652 (1928). Ihre Konstitution ist *A Ar B M z Mp S K* Wb_2 *St.* Kreuzung Nr. 261 wurde ausgeführt zwischen L. 232 und L. 15, die H. u. O. TEDINS Roterbse entspricht; Formel: *A Ar B m F Z oh mp S K* Wb_2 *St.* Und Kreuzung Nr. 262 schliesslich wurde ausgeführt zwischen L. 232 und L. 20, die identisch ist mit H. u. O. TEDINS 0651; Formel: *A Ar B M f z mp S K* Wb_2 *St.* Die drei Linien Nr. 15, 19 und 20 waren stark wachsig. Linie Nr. 232 ist als schwach wachsig zu bezeichnen, jedoch ist die Ausbildung des Wachsüberzuges nicht auf allen vegetativen Teilen der Pflanze gleich schwach. So ist die Oberseite der Blättchen am deutlichsten wachsig, auch der Stengel ist deutlich wachsig. Sehr schwach wachsig sind die Nebenblätter, die Unterseite der Blättchen und die Kelchblätter und ganz wachslos sind die Hülsen. Eine solche nähere Charakteristik der Wachsigkeit fehlt bisher für die verschiedenen Typen; eine solche wäre sehr erwünscht, insbesondere für die verschiedenen, von WELLENSIEK gefundenen Allelen. Dass es sich bei meiner Linie Nr. 232 um das Gen *Wb* handelt, dürfte nicht in Frage gestellt werden können, da es klare Koppelung mit sowohl *S* wie *K* gezeigt hat.

Die F_1-Generationen der genannten drei Kreuzungen waren alle stark wachsig. In keiner dieser Kreuzungen ist Semi- oder partielle Sterilität aufgetreten, alle waren also voll fertil. In F_2 wurden von jeder 500 Samen ausgesät und diese haben folgende Prozente reifer,

in allen Eigenschaften beurteilbare Pflanzen gegeben. Nr. 246 93,0 %, Nr. 261 92,6 % und Nr. 262 94,4 %. Die Keimfähigkeit war demnach durchweg sehr gut, so gut wie man dies von gutem Saatgut einer Sorte verlangen kann. In den drei Genen *Wb*, *S* und *K* wurden folgende Spaltungen beobachtet (die übrigen Gene verbleiben hier unberücksichtigt):

Kreuzung Nr. 246.

339 Wb_2 *S* : 27 Wb_2 *s* : 18 *wb S* : 81 *wb s*; Crossingover = 10,19 %.
306 Wb_2 *K* : 60 Wb_2 *k* : 33 *wb K* : 66 *wb k*; » = 22,54 %.
301 *S K* : 56 *S k* : 38 *s K* : 70 *s k* ; » = 25,30 %.

Kreuzung Nr. 261.

342 Wb_2 *S* : 23 Wb_2 *s* : 33 *wb S* : 65 *wb s*; » = 13,05 %.
321 Wb_2 *K* : 44 Wb_2 *k* : 40 *wb K* : 58 *wb k*; » = 20,13 %.
317 *S K* : 48 *S k* : 44 *s K* : 54 *s k* ; » = 22,41 %.

Kreuzung Nr. 262.

357 Wb_2 *S* : 9 Wb_2 *s* : 13 *wb S* : 93 *wb s*; » = 4,78 %.
331 Wb_2 *K* : 35 Wb_2 *k* : 35 *wb K* : 71 *wb k*; » = 16,13 %.
324 *S K* : 46 *S k* : 42 *s K* : 60 *s k* ; » = 20,78 %.

Die vorstehend mitgeteilten Crossingover-Werte für die Koppelungen zwischen den Genen *Wb*, *S* und *K* sind in Fig. 1 graphisch wiedergegeben.

Die erhaltenen Zahlen für die Koppelungswerte dürften einwandfrei zeigen, dass die drei in Rede stehenden Gene die in Fig. 1 wiedergegebene Anordnung haben: *S*—*Wb*—*K*. Ferner scheinen die gefundenen Werte für einen Wechsel im Koppelungsgrad zu sprechen. Die Werte der beiden Kreuzungen Nr. 246 und 261 stimmen ziemlich gut überein, während die von Kreuzung Nr. 262 deutlich abweichen. Für die Gene *S* und *Wb* passen die Werte gut in die zweite und dritte der früher erwähnten drei Gruppen von Koppelungswerten für diese. Eine Gruppe mit etwa 1—2 %, eine mit ca. 5 % und eine mit 10—12 % Crossingover.

Zu den vorliegenden drei Kreuzungen Nr. 246, 261 und 262 ist zu erwähnen, dass der Chenille-Charakter, *ss*, stets deutlich ausgebildet war, sodass fehlerhafte Beurteilungen als *S* nicht zu befürchten waren. Es wurde also stets gute monohybride Spaltung nach 3 *S* : 1 *s* erhalten. Ferner ist zu erwähnen, dass die Eltern dieser Kreuzungen gleich mittelspät bis spät sind und dass die drei Kreuzungen gleichzeitig und im selben Jahr, 1935, ausgeführt worden sind. Auch die F_1- (und F_2-)

Generationen wurden nebeneinander und unter gleichen Verhältnissen gebaut (1936 bzw. 1937). Nochmals sei hier hervorgehoben, dass alle drei Kreuzungen den einen Elter, L. 232, gemeinsam hatten.

Mit Hinblick auf diese Verhältnisse bin ich der Ansicht, *dass der abweichende Crossingover-Prozent* in Kr. 262, im Vergleich mit 246 und

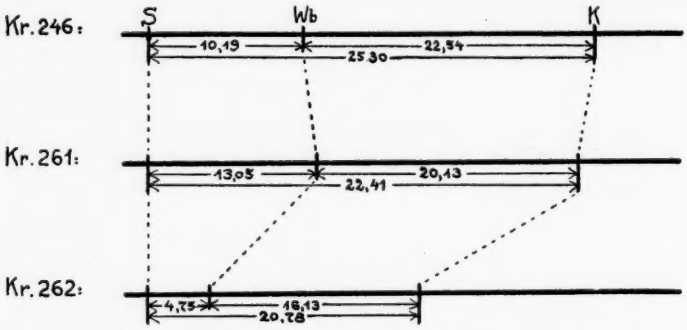

Fig. 1. Graphische Darstellung der Koppelungsverhältnisse zwischen den Genen *S*, *Wb* und *K* in den drei Kreuzungen Nr. 246, 261 und 262 von *Pisum sativum*.

261, *durch verschiedene genotypische Konstitution des zweiten Elters zu erklären ist.*

Diese Verhältnisse erinnern an einen von mir früher für *Phaseolus vulgaris* veröffentlichten Fall (LAMPRECHT, 1932), in dem die drei Gene *Fa*, *Ea* und *Da* in zwei Kreuzungen (Nr. 5 und 12) deutlich verschiedene

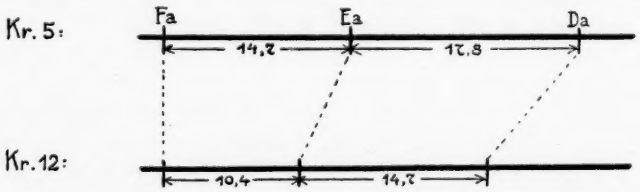

Fig. 2. Graphische Darstellung der Koppelungsverhältnisse zwischen den Genen *Fa*, *Ea* und *Da* in den Kreuzungen Nr. 5 und 12 von *Phaseolus vulgaris*.

Koppelung zeigten. Die in diesen erhaltenen Werte sind hier in Fig. 2 wiedergegeben. Auch damals versuchte ich diese Erscheinung auf verschiedene genotypische Konstitution der Eltern zurückzuführen.

Das Gen *Wa* für Ausbildung von wenig Wachs ist auch in seinen Beziehungen zu *K* untersucht worden. SVERDRUP (1927) fand hierfür freie Kombination mit folgenden Zahlen:

Gefunden:	214	$Wa_2\,K$:	85	$Wa_2\,k$:	63	$wa\,K$:	28	$wa\,k$
Erwartet:	219,37	» :	73,12	» :	73,12	» :	24,37	»
D/m für 9:3:3:1	= — 0,55		+ 1,54		— 1,31		+ 0,76	

Diese Zahlen bilden einen weiteren Beweis dafür, dass *Wa* und *Wb* verschiedene Gene sind, allerdings unter der Voraussetzung, dass an den betreffenden Kreuzungen nicht Linien teilgenommen haben, die in dem *K* bzw. *Wa* enthaltenden Chromosom eine Translokation zeigen, die Koppelung in freie Kombination verwandelt haben könnte.

In seinem Verhalten zu *Wb* wurde *Wa* von WELLENSIEK (1928 a) in zusammen sechs Kreuzungen untersucht. Er fand insgesamt, wie zu erwarten war, voneinander unabhängige Vererbung mit den Zahlen:

Gefunden:	772	$Wa_2\,Wb_2$:	247	$Wa_2\,wb$:	331	$wa \begin{pmatrix} Wb_2 \\ wb \end{pmatrix}$		
Erwartet:	759,38	» :	253,12	» :	337,50	»		
D/m für 9:3:4	= + 0,69		— 0,43		— 0,41			

WELLENSIEK (1925 a und 1928 a) hat auch die Beziehungen des Grundgens für die Ausbildung von Wachs, *Bl*, zu *Wa* und *Wb* in mehreren Kreuzungen untersucht. Für die beiden Gene *Bl* und *Wa* wurde erhalten:

Gefunden:	370	$Bl\,Wa_2$:	106	$Bl \begin{pmatrix} Wa_1 \\ wa \end{pmatrix}$:	131	$bl \begin{pmatrix} Wa_2 \\ Wa_1 \\ wa \end{pmatrix}$		
Erwartet:	341,44	» :	113,81	» :	151,75	»		
D/m für 9:3:4	= + 2,34		— 0,81		— 1,94			

Die Zahlen zeigen eine wahrscheinlich voneinander unabhängige Vererbung der beiden Gene *Bl* und *Wa* an. Für *Bl* und *Wb* wurde in zusammen fünf Kreuzungen erhalten:

Gefunden:	561	$Bl\,Wb_2$:	174	$Bl \begin{pmatrix} Wb_1 \\ wb \end{pmatrix}$:	256	$bl \begin{pmatrix} Wb_2 \\ Wb_1 \\ wb \end{pmatrix}$		
Erwartet:	557,44	» :	185,81	» :	247,75	»		
D/m für 9:3:4	= + 0,23		— 0,96		+ 0,61			

Dieses Spaltungsverhältnis stimmt sehr gut mit bei freier Kombination in *Bl* und *Wb* erwarteten überein.

Das von E. NILSSON (1933) entdeckte Gen *Wlo* wurde von diesem Forscher auch auf seine Beziehungen zu *Wa* und *Wb* hin studiert (1939). Diese drei Gene zeigten voneinander unabhängige Vererbung laut folgenden Zahlenverhältnissen:

Gefunden:	224	*Wa Wb Wlo*	:	63	*Wa Wb wlo*	:	63	*wa Wb Wlo*	:	22	*wa Wb wlo*
Erwartet:	209,25	»	:	69,75	»	:	69,75	»	:	23,25	»
D/m für 9:3:3:1 =	+ 1,54			— 0,90			— 0,90			— 0,27		
Gefunden:	260	*Wa Wb Wlo*	:	103	*Wa Wb wlo*	:	79	*Wa wb Wlo*	:	32	*Wa wb wlo*
Erwartet:	266,62	»	:	88,87	»	:	88,87	»	:	29,62	»
D/m für 9:3:3:1 =	— 0,61			+ 1,67			— 1,16			+ 0,45		

E. NILSSON (1939) konnte das Gen *Wlo* auch mit *P* (das eine Gen für Ausbildung von Membran in der Erbsenhülse) und *Pl* (schwarzes Hilum) in eine sichere Koppelungsgruppe vereinigen. Es wurden mehrere Kreuzungen studiert, die zusammen für diese Koppelungsgruppe das folgende durchschnittliche Bild ergaben: *Wlo* — 12,7 % — *P* — 28,5 % — *Pl*.

LAMM (1937) fand bei seiner Analyse der Gene für Stammlänge bei *Pisum* eine starke Andeutung für Koppelung zwischen *Wlo* und Cy_2. Die von ihm verwendete *wlo*-Linie war identisch mit der von E. NILSSON (l. c.) benutzten.

Selbst habe ich 1938 eine Kreuzung, Nr. 293, zwischen Linie 33, eine Zuckererbse vom Säbel-Typus der Formel *Wa Wb Wlo p v*, und L. 341 (= E. NILSSONs *wlo*-Linie aus English Wonder) in F_2 studiert. F_1 zeigte stark wachsige Pflanzen. F_2 spaltete in 336 *Wlo* : 113 *wlo* mit D/m für 3 : 1 = 0,08. Für die beiden Gene *P* und *Wlo* resultierten folgende Zahlen:

Gefunden:	239	*P Wlo*	:	111	*P wlo*	:	107	*p Wlo*	:	2	*p wlo*
Erwartet:	252,56	»	:	84,19	»	:	84,19	»	:	28,06	»
D/m für 9:3:3:1 =	— 1,29			+ 3,52			+ 2,76			— 5,08		

Es besteht offenbar starke Koppelung. Als Crossingover-Wert ergibt sich 16,72 %, was mit E. NILSSONs Ergebnissen recht gut übereinstimmt.

Fassen wir zusammen, so ist über die Koppelungsverhältnisse der bisher bekannten Gene für die Ausbildung von Wachs bei *Pisum*, *Bl*, *Wa*, *Wb* und *Wlo*, folgendes festgestellt. Das Gen *Wb* gehört zur Koppelungsgruppe *S*—*Wb*—*K*, das Gen *Wlo* zur Koppelungsgruppe *Wlo* —*P*—*Pl*. Die beiden übrigen Gene *Bl* und *Wa* zeigen bei Kreuzung

sowohl untereinander wie mit *Wb* voneinander unabhängige Vererbung. Das Verhalten von *Bl* zu *Wlo* ist noch nicht untersucht worden.

EIN NEUES GEN FÜR LOKALE AUSBILDUNG VON WACHS, Wsp.

Anfang 1936 erhielt ich eine Erbsensamenprobe unter der Bezeichnung »Johnson's British Empire». Das Anbauresultat entsprach indessen keineswegs der Erwartung. Statt nur ganz wachslose Pflanzen, *bl*, zu erhalten, entwickelten sich, abgesehen von drei Pflanzen, lauter stark wachsige. Diese drei Pflanzen zeigten einen mir ganz unbekannten neuen Typus von Wachsigkeit. Die Oberseite der Blättchen war stark wachsig, wie bei Erbsensorten im allgemeinen, aber alle übrigen Teile waren wachslos. Diese Pflanzen zeigten demnach in bezug auf die Ausbildung von Wachs genau die reziproke Verteilung wie E. NILSSONS *wlo*-Pflanzen. Bei diesen ist nämlich nur die Oberseite der Blättchen wachslos, während alle übrigen Teile normale Ausbildung von Wachs zeigen.

Es wurden gleich einige Befruchtungen durchgeführt. Als zweiter Elter wurde u. a. Linie 240, eine niedrige frühe Kneifelerbse benutzt. Diese Kreuzung, Nr. 302, zeigte in F_1 nur stark wachsige Pflanzen. Die 1938 studierte F_2 umfasste insgesamt 146 Individuen und zeigte folgende Spaltung:

115 stark wachsig : 31 nur Oberseite der Blättchen wachsig.

D/m für monohybride Spaltung = 1,05, also gute Übereinstimmung anzeigend. Das für diese Spaltung verantwortliche Genpaar will ich, abgeleitet von *W* (Wachs) *p*ars *s*uperior (= Oberseite), mit *Wsp—wsp* bezeichnen. Die Formel des neuen, lokal wachsigen Typus (L. 360) ist wahrscheinlich *Bl Wa Wb Wlo wsp*.

Einstweilen erscheint es natürlich nicht sicher ob diese Bezeichnung beibehalten werden kann, da es ja denkbar wäre, dass es sich um ein Allel zu *Wlo* handelt. Kreuzungen zur Beantwortung dieser Frage sind bereits ausgeführt.

SUMMARY.

1. The effect of the previously known genes for the formation of wax on the vegetative parts of *Pisum*, *Bl*, *Wa*, *Wb* and *Wlo*, is discussed comprehensively.

2. The linkage relations between these genes and others, with

which they show linkage, are comprehensively treated and new segregation numbers are recorded.

3. The location of the genes belonging to the linkage group *S—Wb —K* is analysed.

4. The occurrence of undoubtedly divergent crossing-over values is demonstrated and is explained by the different genotypic constitution of the parents.

5. A new gene for the local development of waxiness, *Wsp* (from *w*ax *p*ars *s*uperior), is demonstrated. In *wspwsp*-plants only the upperside of the leaflets is intensely ceraceous, the other parts being non-ceraceous.

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NOTES ON THE CHROMOSOMES OF SOME TELEOSTS

ESOX LUCIUS L., LUCIOPERCA LUCIOPERCA L. AND PERCA FLUVIATILIS L.

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I. INTRODUCTION, MATERIAL AND METHODS.

IN 1932 OGUMA and KAKINO published a check-list of the chromosome number in vertebrates, covering investigations up to the beginning of that year. As far as we have been able to find the list is complete with reference to Teleosts, but since it was published, some further papers have appeared dealing with fish chromosomes (FRIEDMAN and GORDON, 1934; MAKINO, 1933 and 1937; PROKOFIEVA, 1934 a and 1934 b; RALSTON, 1933). But still the number of Teleosts cytologically investigated is not higher than 22. Only few of these are of recent date. Thus, as far as the chromosomes are concerned the Teleosts are the most incompletely known group of vertebrates. On account of their low systematic position they probably conceal several interesting problems and therefore it is highly desired to study them more closely.

Our material comprised embryos 4 to 5 days of age, prepared out of the yolk, fixed and stained in BELLING's iron aceto-carmine for 24 hours. We attached great importance to a fast work in order to avoid post-mortem changes in the tissues. Then smears were made in the manner described in BOLLES LEE, 1937, p. 273. Suitable metaphase plates were selected and drawn. The optic used was a ZEISS's binocular microscope with apochromatic objective 90, apert. 1,30 and eyepieces 15x and 20x. Camera lucida was not used.

A great number of metaphase plates were studied for each species, but only the best were drawn (*Esox* 9, *Lucioperca* 6 and *Perca* 6 plates). The chromosome numbers in all plates coincided exactly for each species.

We chose embryonic material in order to get clear views of the appearance of the chromosomes, which, as is well known, are not so contracted at mitotic metaphase as at meiosis. Embryonic material was successfully used by MAKINO (1937) and PROKOFIEVA (1934 a, b).

But they worked with sections and not with smears. The smear method gives results quite as good as sections and is incomparably more handy.

II. RESULTS.

1. Esox lucius L. (Figs. 1 and 2.) — The diploid number of chromosomes in this fish was determined at 18, made up of 9 pairs. There are 4 pairs of long V-shaped chromosomes, one pair of long J-shaped chromosomes, one big and two smaller pairs of rod-shaped and finally one pair of small V-shaped chromosomes. With the exception of the long rod-shaped ones, these types correspond to PROKOFIEVA's (1934 a, b) types found in embryos of Salmonidae. The same types MAKINO (1937) also found in the Salmonid fish *Oncorhynchus Keta* WALB.

As regards the arrangements of the chromosomes in the plates we may mention that one of the big V-shaped chromosomes always lies in the middle, more or less deformed. The deformations probably owe their origin to secondary constrictions. However, we have not made any measurements of the exact localities of the constrictions in the chromosomes. In examining metaphase plates of *Esox,* it is striking that chromosomes belonging to the same pair often lie immediately beside each other. This fact is most obvious in the case of the biggest pair of V-shaped chromosomes, which in all plates we examined lie side by side. In one plate (Fig. 1), the chromosomes in every pair lie together, with the exception of the long J-shaped ones. We intend to make further studies of this somatic pairing.

In none of the embryos examined were we able to discover anything indicating the presence of sex-chromosomes, which is in accordance with the results of most earlier cytological investigators of fish chromosomes.

2. Lucioperca lucioperca L. (Figs. 3 and 4.) — The metaphase plates of the Swedish pike-perch consist of 24 chromosomes, i. e. 12 pairs. The types found here are 4 pairs of long V-shaped, one pair of long J-shaped, one pair with subterminal centromeres, one pair long rod-shaped and ten small chromosomes. The chromosome types described by PROKOFIEVA and MAKINO from Salmonidae are thus also found here.

In *Lucioperca,* too, it is evident that chromosomes belonging to the same pair are often found beside each other, though it is not so clear in this species as in the other two investigated.

We did not find anything indicating the presence of sex-chromo-

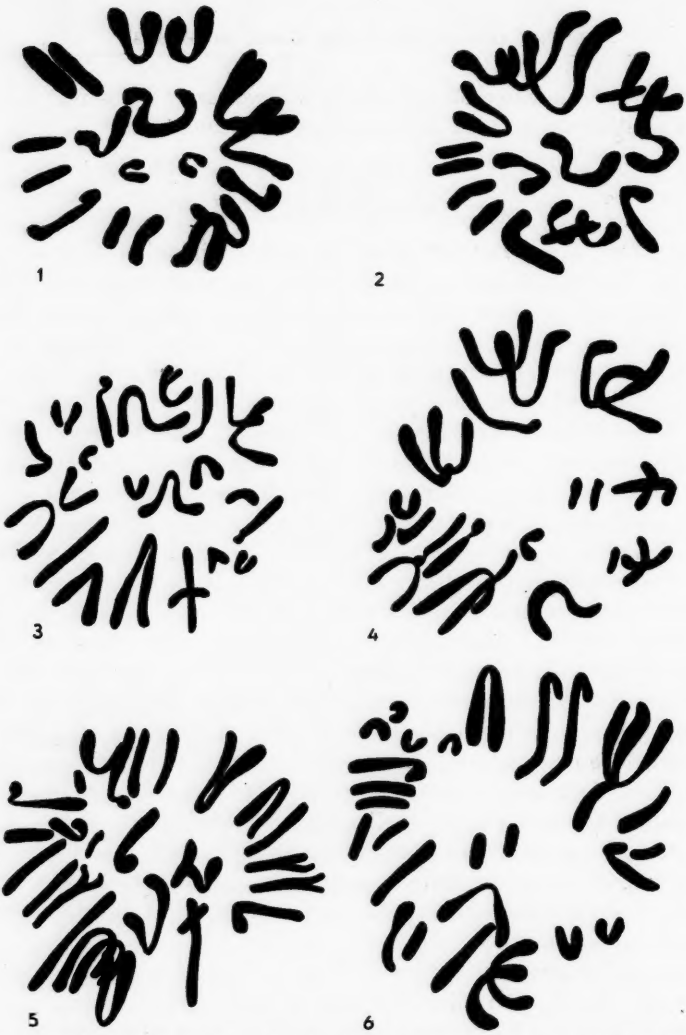

Fig. 1. Metaphase plate of *Esox* showing the 18 chromosomes with the members of each pair lying side by side, with the exception of the J-shaped. One of the big V:s to the right of the plate seems to be divided at one end, as the chromatids do not keep quite together. — Fig. 2. Metaphase plate of *Esox*. The pairing of the 18 chromosomes is not so complete as in Fig. 1, but the members of 4 pairs lie together. — Fig. 3. Metaphase plate of *Lucioperca* with the 24 chromosomes. — Fig. 4. Metaphase plate of *Lucioperca* showing 24 chromosomes. 5 pairs lie together. — Fig. 5. Metaphase plate of *Perca* with its 28 chromosomes. Several pairs keep together. 3 rod-shaped chromosomes are divided in the same manner as the »V» in Fig. 1. — Fig. 6. Metaphase plate of *Perca* showing the members of almost all 14 pairs lying side by side with their partners.

somes. At least such chromosomes are not to be found among the seven largest pairs.

3. Perca fluviatilis L. (Figs. 5 and 6.) — The diploid number found here is 28. This number is in rather good agreement with that found by TURNER (1919), who stated that the diploid chromosome number of »perch» was 27. He says nothing about the species used for his investigation (the only mention made in his paper is the English specific name »perch»), but, as far as we know, the North American perch differs very little from the Swedish and is systematically considered as a subspecies, *P. fluviatilis* flavescens* BERG.

TURNER, however, worked with testes and if the number 27 found by him is correct, it would seem to indicate that the male perch should be of the type XO. He further states that he found nothing in the anaphase behaviour of the chromosomes, such as lagging etc., which is usual for sex-chromosomes in XO species. And, as no other investigator has found cytological evidences of XO types in Teleosts, TURNER's chromosome number is probably not correct.

TURNER's drawing from a spermatogonium shows small dot-shaped chromosomes. We found 3 pairs of long V-shaped, 2 pairs of long J-shaped and 9 pairs of more or less long, rod-shaped chromosomes, i. e. also in this species the same types found by MAKINO and PROKOFIEVA.

The behaviour of the chromosomes in the metaphase does not differ from that reported for the two other species and will, as already mentioned, be the subject of further investigations.

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SUMMARY.

1. The diploid number of chromosomes in *Esox lucius* L. is found to be 18. There are 6 pairs of long chromosomes, among which 4 pairs are V-shaped.

2. In the Swedish pike-perch, *Lucioperca lucioperca* L., the diploid number is 24. 7 pairs are long and in this species, too, there are, among the long chromosomes, 4 pairs V-shaped ones.

3. The perch, *Perca fluviatilis* L., is found to have a diploid chro-

mosome number of 28. 5 pairs are long, 9 pairs, mostly rod-shaped, are smaller. Among the biggest there are only 3 pairs of V-shaped chromosomes.

4. Somatic pairing, that is, the members of every chromosome pair lying more or less together, is found especially in *Esox* and *Perca*. This will be the subject of further studies.

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STUDIES IN DROSOPHILA MELANOGASTER WITH ATTACHED X:S

II. FREQUENCY OF HOMOZYGOSIS

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I. INTRODUCTION.

THE study of *Drosophila* with attached *X* chromosomes has enabled a number of problems to be attacked, which would otherwise have been difficult to get at. One of these problems is that of the chromatid interference, i. e. the question as to whether the chromatids exchanging parts at a certain chiasma have an influence on the determination of what chromatids will exchange parts at distally situated chiasmata (BEADLE and EMERSON, 1935; MATHER, 1935, 1938; WEINSTEIN, 1936; BONNIER and NORDENSKIÖLD, 1937). An especially practicable method in this respect has been the study of the percentage of homozygous daughters, which daughters emerged after heterozygous mothers. As a rule the frequencies of homozygosis found were compared with those expected if no interference had been present. When no reliable differences were obtained the conclusion was drawn that no chromatid interference had taken place, while in the contrary case such an interference must have been present. In this paper an attempt will be made to define more in detail those causes which influence the determination of the frequency of homozygosis, and also to deduce the applicable formula. Further, an account will be given of an experiment performed for the purpose of increasing this frequency by selection, and, finally, the progression of such experiments as should furnish valuable information in this field is outlined.

II. FORMULA FOR THE FREQUENCY OF HOMOZYGOSIS.

The formula for the frequency of homozygosis has been deduced by several authors (SAX, 1932; BELLING, 1933; KIKKAWA, 1933; MATHER, 1935; BEADLE and EMERSON, 1935; WEINSTEIN, 1936), it being assumed that no chromatid interference was present. WEINSTEIN discussed in

detail the result following different kinds of divergences from random chromatid associations, but no formula was computed.

The frequency of homozygosis is determined by a number of different causes. In order to give an account of these causes we shall make certain restrictions with regard to the complete general applicability of the hypotheses. But these restrictions are partly in accordance with the present general conceptions of the nature of the crossing-over phenomenon (compare, for instance, MATHER, 1938, and DARLINGTON, 1939) and partly we shall in each separate case call attention to the significance of these restrictions. [Unfortunately different authors employ quite different nomenclatures. In the present paper the same nomenclature will be employed as in our previous article (BONNIER and NORDENSKIÖLD, 1937). Each time an expression is employed below for the first time synonyms, if they exist, will be given in brackets.]

We presume that crossing-over takes place in connexion with a division of the chromosomes into chromatids during pachytene, and that a proximal (counting from the centromere) crossing-over precedes in time distal crossings-over. In that way we may introduce the probability that an exchange of a certain kind occurs at a certain chiasma provided one knows the nature of the chiasma situated most proximally to it. The restriction we thus introduce is that the possibly occurring chromatid interference is only effective from one chiasma to the nearest, distally situated, chiasma, but not to chiasmata situated still more distally. At the same time we presume that no crossing-over occurs between sister chromatids. In view of the fact that the incidence of 4 chiasmata or more is very rare in *Drosophila*, we confine our calculations to cases with at most 3 chiasmata per chromosome.

The first chiasma, i. e. the one situated nearest the centromere, may be of two kinds, non-reciprocal or reciprocal [between homologous unattached or homologous attached chromatids (WEINSTEIN, 1936)]. Let k denote the relative frequency of the first chiasmata which are non-reciprocal. The frequency of homozygosis for loci situated between the first and the second chiasma is determined by the value of k, and in that way this value will also be co-determinative for the frequency of homozygosis for loci situated distally to the second chiasma. These frequencies are influenced besides by the chiasma frequency and the chromatid interference proper. The relation between the first and the second chiasma may be such that (1) the same two chromatids participating in the first chiasma also take part in the second chiasma, (2) only one of the chromatids taking part in the first chiasma participates in

the second, or (3) the two chromatids participating in the first chiasma do not take part in the second. In accordance with BEADLE and EMERSON (1935) we call these three different forms of relations (named in the same order) 2-strand double, 3-strand double, 4-strand double [regressive, progressive, digressive (WEINSTEIN, 1936); reciprocal, diagonal, complementary (cf. DARLINGTON, 1937)]. In the material studied in our preceding article it was shown that these 3 cases were not of equal frequency. Call the relative frequency of the 3 cases (still in the same order) c_2, $2c_3$ and c_4, thus $c_2 + 2c_3 + c_4 = 1$. (That called $2c_3$ here was called c_3 in our previous paper.)

When a third chiasma occurs we assume that its nature may be dependent on the conditions in the second chiasma, but, as mentioned above, not on the conditions in the first chiasma. Therefore if we regard the chromatids from the point of view of the relations existing between their No. 2 and No. 3 chiasmata, they may likewise be 2-strand, 3-strand and 4-strand double. Call the relative frequency of these d_2, $2d_3$ and d_4 ($d_2 + 2d_3 + d_4 = 1$). By making the hypotheses described here we have not only made the restriction in the general applicability that the chromatid interferences may be able to act from a given chiasma only to the next chiasma situated distally to it, for we have also made the restriction contained in the assumption that the magnitude of the chromatid interference (i. e. the values c and d) is fixed by our knowledge of the *ordinal number* of the chiasma. Of course the magnitude of the interference may — and in fact this seems to be more probable — be determined by the distance between the two successive chiasmata. But in view of the localization of the chiasmata, which seems to be a general rule (MATHER, 1936, 1937, 1938), it is probable that this does not imply any serious restriction to the general applicability.

Guided by the hypotheses made — which thus restrict the general application to some extent, although probably not very much — the frequency of homozygosis will be determined by (1) the frequency of non-reciprocal first chiasmata (the number k), (2) the magnitude of the chromatid interference (the numbers c and d), and (3) the chiasma frequency.

The calculations are made in the same manner as in the above-mentioned works when only random associations occur (in which case $k = 1/2$ and all c and $d = 1/4$). By working through all conceivable cases one may be convinced that the relation between the number of

chiasmata and the relative frequency of chromatids that are non-cross-overs, single, double and triple cross-overs will be the following:

No. of chiasmata	Chromatids that are cross-overs			
	0	1	2	3
0	1			
1	$^1/_2$	$^1/_2$		
2	$\frac{c_2+c_3}{2}$	c_3+c_4	$\frac{c_2+c_3}{2}$	
3	$\frac{(c_2+c_3)(d_2+d_3)}{2}$	$\frac{(c_2+c_3)(d_3+d_4)+c_3+c_4}{2}$	$\frac{(c_2+c_3)(d_3+d_4)+c_3+c_4}{2}$	$\frac{(c_2+c_3)(d_2+d_3)}{2}$

If the relative frequencies of tetrads with 0, 1, 2, 3 chiasmata are called x_0, x_1, x_2, x_3 and the relative frequencies of found chromatids with 0, 1, 2, 3 cross-overs m_0, m_1, m_2, m_3 the following equations will obviously be obtained:

$$(1)\ \ldots \begin{cases} m_0 = x_0 + \frac{x_1}{2} + \frac{c_2+c_3}{2}x_2 + \frac{(c_2+c_3)(d_2+d_3)}{2}x_3 \\ m_1 = \frac{x_1}{2} + (c_3+c_4)x_2 + \frac{(c_2+c_3)(d_3+d_4)+c_3+c_4}{2}x_3 \\ m_2 = \frac{c_2+c_3}{2}x_2 + \frac{(c_2+c_3)(d_3+d_4)+c_3+c_4}{2}x_3 \\ m_3 = \frac{(c_2+c_3)(d_2+d_3)}{2}x_3 \end{cases}$$

Since $c_2 + 2c_3 + c_4 = d_2 + 2d_3 + d_4 = 1$, all c and all d are not independent of each other. Therefore the following two new expressions may be introduced:

$$s = \frac{c_2+c_3}{2} \text{ and } t = \frac{d_3+d_4}{2}$$

Then the values of x_1, x_2 and x_3 will be as follows:

$$(2)\ \ldots \begin{cases} x_1 = 2m_1 - \frac{2(1-2s)}{s}m_2 + \frac{4st-2s+1}{s^2(1-2t)}(1-3s)\ m_3 \\ x_2 = \frac{m_2}{s} - \frac{4st-2s+1}{2s^2(1-2t)}m_3 \\ x_3 = \frac{m_3}{s(1-2t)} \end{cases}$$

If no chromatid interference is present $s = t = {}^1/_4$ and then the usual expressions are obtained:

$$x_1 = 2m_1 - 4m_2 + 6m_3$$
$$x_2 = 4m_2 - 12m_3$$
$$x_3 = 8m_3$$

Finally, as regards the frequencies of homozygosis it is possible by working through all conceivable cases to show that these are:

The relative frequency of recessive (or dominant) homozygotes after

$$(3) \ldots \begin{cases} 1 \text{ chiasma} & H_1 = \dfrac{k}{2} \\ 2 \text{ chiasmata} & H_2 = (1-k)\ c_3 \\ 3 \text{ chiasmata} & H_3 = (1-k)\ d_3\ (1-2c_3) + \dfrac{k}{2}\ (1-2d_3) \end{cases}$$

When $k = {}^1/_2$ and $c = d = {}^1/_4$ we obtain the usual values

$$H_1 = {}^1/_4,\ H_2 = {}^1/_8,\ H_3 = {}^3/_{16}$$

If the frequency of recessive homozygotes found is called H, it is obvious that

$$(4) \ldots\ldots\ldots\ldots\ldots H = H_1 x_1 + H_2 x_2 + H_3 x_3,$$

into which expressions the individual frequencies of homozygosis H_1, H_2, H_3 from (3) and the chiasma frequencies x_1, x_2, x_3 from (2) are to be introduced. Of the five parameters, k, s, t, c_3 and d_3, entering into the expression (4), k, c_3 and d_3 are at the lowest 0 and at most 1 and s and t at the lowest 0 and at most ${}^1/_2$. If they differ widely from the values applicable when only random associations are present ($k = {}^1/_2$, the others $= {}^1/_4$), the frequency of homozygosis may obviously assume any value. For instance, if $k = 0$ (only reciprocal first chiasmata) and also $c_3 = d_3 = 0$ (no 3-strand doubles) the frequency of homozygosis will be $= 0$. On the other hand, if $k = 0$, $c_3 = 0$, $d_2 + d_3 = 2m_3$ and $c_2 = d_3$ the frequency of homozygosis will be $= 1$. If the frequency of homozygosis can be altered by means of selections in such a manner that this frequency assumes values differing from those usually found, it seems to be just as probable that that may be due to values of the parameters changed by the selection as due to a change in the chiasma frequency.

In order to get an idea as to whether a certain percentage of homozygosis is compatible with the assumption that the entire change is due to a change in the chiasma frequency, we may proceed in the

following manner. If the normal values of H_1, H_2 and H_3 are introduced into equation (4), this equation may be written as follows:

(5) ..... $4x_1 + 2x_2 + 3x_3 = 16\,H$

Moreover,

(6) ..... $x_1 + x_2 + x_3 \leq 1$

Two of the numbers x may be eliminated from (5) and (6) and we then obtain the following 3 differences:

(7) ..... $x_1 \geq 8\,H - 1 - \frac{x_3}{2}$

(8) ..... $x_1 \geq 16\,H - 3 + x_2$

(9) ..... $x_2 \leq 2 - 8\,H - \frac{x_3}{2}$

Since none of the numbers x can be negative and at most $= 1$, the minimum value of x_1 will be equal to the larger of $8\,H - 1{,}5$ or $16\,H - 3$. The maximum value of x_1 is obtained from (5) by making $x_2 = x_3 = 0$, i. e.

(10) ..... $x_1 \leq 4\,H$

III. SELECTION OF HIGH PERCENTAGE OF HOMOZYGOSIS.

In order to facilitate the work of selection as much as possible only one gene was taken into consideration, viz. the gene *y* for yellow body colour situated in the distal end of the *X* chromosome. A female (with attached *X* chromosomes), which was heterozygous with respect to *y* was mated with a Bar male on 19th February, 1935, and gave 94 daughters, 23 of which were homozygous *y*, i. e. a percentage of homozygosis of 24,47. 9 of her daughters were mated in separate cultures with full brothers (as a rule no pair cultures were made, each culture being composed of 1 female and generally 3 of her full brothers. This procedure was also adopted in subsequent matings. All cultures were raised at 25° C.). These daughter cultures constituted generation No. 1, and from them were hatched altogether 863 daughters, 165 of which were *y*, i. e. a percentage of homozygosis of 19,12. In the incubation of these cultures the count was put off for some days (generally 4 days from the beginning of the hatching), and from some of those cultures showing the highest percentage of homozygosis females were again taken which were mated in separate cultures with full brothers, which gave rise to generation No. 2. The cultures were continued in this manner until altogether 17 generations

were obtained, and during this sequence of generations the average percentage of homozygosis increased, as shown in Table 1, to more than 22. (Here and in subsequent computations females giving fewer than 30 daughters have been excluded. Detachment females have not been included among the daughters, nor have they been included in the calculations of the percentages of homozygosis.)

TABLE 1. *Showing the effect of selection.*

Generation	+	*y*	Detachements	Total (except detachements)	Per cent. homozygosis	Total number of cultures	Cultures from which daughters were taken to make up cultures of next generation	
							Number of cultures	Average percentage of homozygosis
1	698	165	0	863	19,12	9	2	26,25
2	359	84	0	443	18,96	4	2	21,50
3	1208	284	0	1492	19,03	18	2	22,82
4	2216	508	7	2724	18,65	34	3	25,64
5	2473	601	0	3074	19,55	32	4	25,62
6	2654	647	1	3301	19,60	41	3	24,83
7	1277	325	0	1602	20,29	22	6	24,06
8	1961	507	3	2468	20,54	38	2	25,17
9	1225	315	1	1540	20,45	22	2	24,82
10	1046	270	1	1316	20,52	21	2	32,50
11	243	54	0	297	18,18	7	3	21,43
12	861	234	0	1095	21,37	21	3	28,42
13	830	295	0	1125	26,22	22	5	26,62
14	1409	425	0	1834	23,17	32	3	30,97
15	677	192	0	869	22,09	16	4	25,37
16	1125	323	0	1448	22,31	25	5	30,00
17	1100	316	0	1416	22,32	28	20	22,87
Total	21362	5545	13	26907	20,61	392	52 [1]	25,69 [1]

After the 17th generation the work was continued along the same lines, but with this difference that no selection was undertaken. Already in the hatchings of the first days (thus before anything could be known of the percentage of homozygosis in the different cultures) daughters were taken from 20 different cultures of generation No. 17 and mated in separate cultures with whole brothers, thereby giving rise to generation No. 18. Cultures were continued — i. e. *with* inbreeding

[1] Except generation 17 but original culture included.

TABLE 2. *Showing generations without selection.*

Generation	+	*y*	Detachements	Total (except detachements)	Per cent. homozygosis	Total number of cultures	Cultures from which daughters were taken to make up cultures of next generation	
							Number of cultures	Average percentage of homozygosis
18	1070	354	0	1424	24,86	27	21	23,34
19	886	262	0	1148	22,82	21	19	21,84
20	1115	341	0	1456	23,42	29	17	23,07
21	741	202	0	943	21,42	17	15	21,12
22	782	237	0	1019	23,26	21	13	23,58
23	620	159	0	779	20,41	15	8	20,63
24	307	103	0	410	25,12	9	13	33,75
25	718	202	0	920	21,96	17	17	21,17
26	798	248	1	1046	23,71	21	15	23,82
27	668	204	1	872	23,39	16	10	24,63
28	411	117	0	528	22,16	12	11	25,90
29	449	140	1	589	23,77	13	7	20,00
30	208	56	0	264	21,21	7	8	24,53
31	349	85	0	434	19,59	9	6	21,51
32	270	76	0	346	21,97	8	4	18,11
33	137	30	0	167	17,96	4	4	20,27
34	384	80	0	464	17,24	9	8	20,06
35	989	311	0	1300	23,92	25	9	20,47
36	499	157	0	656	23,93	15	11	23,58
37	408	114	0	522	21,84	13	5	24,14
38	143	46	0	189	24,34	5	9	28,93
39	443	130	0	573	22,69	11	9	22,83
40	1749	458	0	2207	20,75	41	7	20,40
41	317	75	0	392	19,13	7	4	19,39
42	207	61	0	268	22,76	6	4	21,94
43	200	63	1	263	23,95	6	4	23,89
44	264	90	1	354	25,42	7	9	25,78
45	310	85	0	395	21,52	8	4	18,80
46	143	40	0	183	21,86	4	5	18,92
47	154	40	0	194	20,62	5	7	26,36
48	247	62	0	309	20,06	8	8	16,53
49	291	76	0	367	20,71	9	6	19,38
50	233	73	0	306	23,86	7	7	24,32
51	323	98	0	421	23,28	10	5	25,83
52	177	50	0	227	22,03	5	4	20,93
53	144	41	0	185	22,16	5	3	22,54
54	105	21	0	126	16,67	3	2	40,00
55	80	20	0	100	20,00	2	2	21,54
56	61	12	0	73	16,44	2	1	13,33
57	27	7	0	34	20,59	1	2	21,05
58	74	29	0	103	28,16	3	1	22,22
59	30	10	0	40	25,00	1	2	25,40
60	59	18	0	77	23,38	2		
Total	17590	5083	5	22673	22,42	466	346 [1]	23,16 [1]

[1] Generation 17 included.

but *without* selection — until a further 43 generations were grown, consequently up to generation No. 60 (Table 2). The percentage of homozygosis had by then become relatively stabilized, and in the 466 cultures comprising these 43 generations altogether 22673 daughters were obtained, 5083 of which were *y*, i. e. an average percentage of homozygosis of 22,42. A great variation exists, however, between the percentages of the different cultures. If these 466 cultures are classified with respect to their percentage of homozygosis, with a class range of 2,5 per cent. and taking as our starting-point the value of 22,4, we obtain the frequency curve shown in Fig. 1. The mean value will then be 22,35 per cent. and the standard error 0,28 per cent. (In these com-

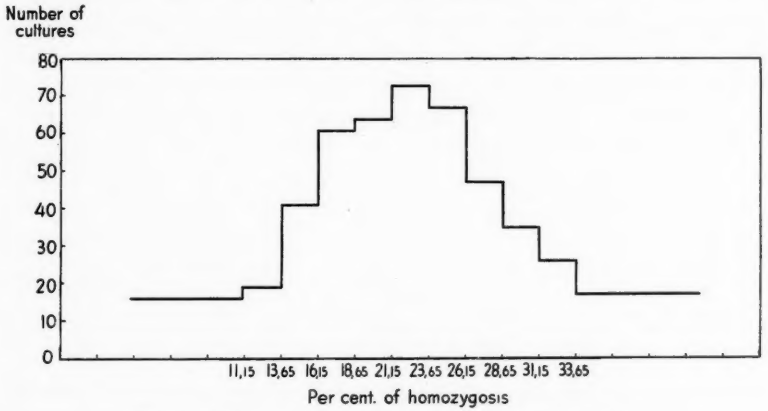

Fig. 1. Frequency curve. Percentage of homozygosis.

putations no allowance has been made for the varying number of animals in the different cultures, all cultures being regarded as equal. The lowest class has been pooled and consists of all cultures having a lower percentage of homozygosis than 11,15. Similarly, the highest class comprises all cultures with a higher percentage of homozygosis than 33,65.)

With the increasing number of generations the average number of daughters per culture decreased, probably due to the intensive inbreeding. Many dead larvae and pupae were also seen. No estimations or studies were made of the mortality. There is a certain possibility that this mortality was selective, and then if the rate of mortality was higher among the wild-type than among the *y* daughters a seemingly increased percentage of homozygosis would be the result. But in that

case cultures with a greater number of daughters should on an average have a lower percentage of homozygosis. In the case of the generations without selection (No. 18—No. 60) the cultures were therefore classed with respect to the number of daughters (Table 3). No tendency to a decreased percentage of homozygosis with increasing numbers of daughters seems to exist. Another possible explanation of the high percentage of homozygosis is that in the progress of the work a lethal gene arose by mutation close to the locus for yellow, but in the chromosome carrying $+^y$. In that way all wild-type homozygotes would disappear, resulting in an apparent increase of the *y* homozygotes. But among the males from the 18th—60th generations 13 detachments

TABLE 3. *Showing the percentage of homozygosis in cultures with different numbers of daughters (Generations 18—60 from Table 2).*

Number of daughters per culture	Number of daughters			Percentage of homozygosis
	+	*y*	Total	
30—39	3806	1094	4900	22,33
40—49	4491	1296	5787	22,40
50—59	4077	1142	5219	21,88
60—69	3163	985	4148	23,75
70 and more.....	2053	566	2619	21,61
Total	17590	5083	22673	22,42

occurred, 7 of which were wild-type and 6 yellow. We should thus be justified in assuming that the increase in the percentage of homozygosis, produced by this experiment in selection, is not apparent but real.

If no changes in the values of the parameters have been produced by the selection performed, the entire effect of the selection being the change in the chiasma frequencies x_1, x_2, x_3, then according to (7), (8) and (10) the minimum and maximum values of x_1 will be 0,5872 and 0,8968, and from (9) we find that $x_2 \leq 0{,}2064 - \frac{x_3}{2}$, thus the maximum value of $x_2 = 0{,}2064$. In the classification made by MATHER (1938, p. 280) the following values were found: $x_1 = 0{,}4849$, $x_2 = 0{,}4289$, $x_3 = 0{,}0302$. Thus if the effect of selection had exclusively affected the chiasma frequency, this effect must have been exceedingly strong.

STEINBERG (1936) has shown that by introducing inversions into the autosomes it is possible to increase the crossing-over frequency in *X*. This increase, however, chiefly affected the frequency of double

and also of triple cross-overs, which — provided only random chromatid associations are present — is easily instrumental in reducing the frequency of single chiasmata to such an extent that the frequency of homozygosis is also decreased. In Table 4 are grouped the values of the chiasma frequencies x_1, x_2, x_3 and x_4, obtained by him (STEINBERG, 1936, Table 4) as well as the frequencies of homozygosis H calculated from them. Thus even if inversions had occurred in the autosomes (which might have been retained in a heterozygous condition if they had been supplemented with a system of balanced lethals), it is hardly probable that the changes in the chiasma frequencies caused

TABLE 4. *Showing the effect (under the assumption of random chromatid associations) of the inversions Payne (Chromosome 2) and C_y (Chromosome 3) on the frequency of single (x_1), double (x_2), triple (x_3) and quadruple (x_4) chiasmata. [Figures from* STEINBERG, *1936, Table 4, and the frequency of homozygosis (H) calculated from it].*

	$\frac{+}{+};\frac{+}{+}$	$\frac{+}{+};\frac{+}{C_y}$	$\frac{+}{\text{Payne}};\frac{+}{+}$	$\frac{+}{\text{Payne}};\frac{+}{C_y}$
x_1	0,689	0,624	0,444	0,164
x_2	0,162	0,316	0,452	0,596
x_3	0,014	0,008	—	0,192
x_4	—	—	0,016	—
H	0,1951	0,1970	0,1700	0,1515

by them would alone have been able to explain the increase in the frequency of homozygosis.

We thus seem to be justified in asserting that even if the percentage of homozygosis of 22,42 is not absolutely incompatible with the assumption that only random chromatid associations are present, nevertheless the correctness of this assumption is hardly probable.

IV. THE USE OF THE FORMULAE.

Most of the classifications made by different authors (v. MATHER, 1938, where classifications are given from different publications) indicate that only random chromatid associations generally occur. But the experiment described in the present article shows that by means of selection one may with a great degree of probability be able to produce changes in this respect. In order to obtain a more thorough knowledge of these problems a systematic work of selection, with selections in different directions, should therefore be carried out.

In order to study the different values that k (the relative frequency of non-reciprocal first chiasmata) may assume, one should employ 2 genes, a, b, which are situated so proximally that it can be assumed that no chiasmata occur between the centromere and a, and that at most one chiasma may occur between a and b. [As a the gene *bb* (bobbed) may be appropriately chosen and as b the gene *car* (carnation)]. Heterozygous $ab/+\ +$ females [or $a+/+\ b$; compare the discussion on this point in BONNIER and NORDENSKIÖLD (1937)] are crossed. From the females giving a high or low percentage of homozygosis for b all daughters are outcrossed. The genetic constitution the daughters then show themselves to possess will indicate whether the mother gave rise to a high or low frequency of cross-over chromatids. The work may afterwards be continued with those combining a high percentage of homozygosis with a low percentage of cross-overs or vice versa. When at most *one* chiasma is present then, according to formula (4)

$$(11) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad k = \frac{H}{m_1}$$

Thus if selections are successful, k may be altered to assume values above and below $^1/_2$.

One should have introduced from the beginning not only the genes a and b but also two other genes (c and d), but at first attention should only be devoted to the conditions in the genes a and b (although of course all classifications must be performed). If — and when — one has succeeded in producing lines with different k-values one should concentrate on selections in c and d. These new genes should be chosen in such a manner that in the first place at most one chiasma can occur between b and c or between c and d and in the second place that at most 2 chiasmata may be expected to occur between the centromere and d [d should therefore not be situated more distally than v (vermilion)]. In the event of at most 2 chiasmata occurring, formula (4) may be written thus:

$$(12) \quad \ldots\ldots\ldots\ldots \quad \frac{H - k\,m_1 - 2\,k\,m_2}{m_2} = \frac{1}{s}\left[c_3\,(1-k) - k\right]$$

If the values of both s and c_3 correspond to only random chromatid associations ($s = c_3 = {}^1/_4$), the expression on the right of equation (12) will be $= 1 - 5\,k$. Subsequent selection work (i. e. in the event of lines having been obtained in which one has succeeded in fixing $k \neq {}^1/_2$)

should therefore be directed in such a manner that — with the values of k for the respective lines — the left side of (12) is made greater or smaller than 1—5 k. If this is done with respect to gene c, and one then goes through the material of a line in which this selection has had an effect, and insert in the left side the values of H, m_1 and m_2 corresponding to gene d, one will be able to find out whether the left side of (12) has the same value for gene d as for gene c. If that is not so, the simplifying hypothesis advanced by us that the chromatid interference is only due to the ordinal number of the chiasmata and not to the distance between them does not hold good.

The investigation may be continued still further to apply to genes situated so far distally that 3 chiasmata may occur proximally to them. In that case the whole of formula (4) must be employed for analysis as the work proceeds. It is probable, however, that this would render the technical difficulties of the work extremely complicated, which will of course be very great nevertheless. One difficulty in particular that should be pointed out is that if the vitality is reduced by the necessary inbreeding in connexion with the selection, and therefore large numbers of daughters cannot be expected in the cultures, the identification of the genetic constitution of the daughters will frequently be doubtful. Moreover, it is to be desired that the salivary gland chromosomes are controlled as often as possible with regard to any inversions that may occur in the X chromosomes as well as in the autosomes. (This was not done in our work with the y homozygosis.) According to STEINBERG (1936), autosomal inversions may change the chiasma frequencies in the X chromosome. His estimations are based, however, on the assumption that only random chromatid associations are present. Therefore in order to obtain a satisfactory idea of the effect of such inversions, the work of selection outlined in this section should be repeated after the introduction of known inversions into chromosomes II and III.

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V. SUMMARY.

1. Formulae for the frequencies of chiasmata and homozygosis are deduced from certain general conditions relating to the nature of the chromatid interference, and the nature of the first chiasma situated distally to the centromere [Formulae (2), (3), (4)].

2. A selection experiment is described, in which the frequency of

homozygosis in the distal end of the *X* chromosome was increased to 22,42 per cent. It is shown that it is very improbable that this frequency was due only to a change in the chiasma frequency, it being therefore more probable that the parameters determining the chromatid interference and the nature of the first chiasma were also changed by the selection.

3. The progression of a detailed experiment is outlined, by means of which it should be possible, with the aid of the computed formulae, to obtain a thorough knowledge of the chromatid associations possible in different lines.

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NOTE ON SOME COLCHICINE-INDUCED POLYPLOIDS

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LAST summer sixteen different plant species were treated with colchicine in order to induce polyploidy. These species were the following: *Secale cereale*, *Triticum vulgare*, *Hordeum vulgare*, *Lolium perenne*, *Festuca pratensis*, *Phleum pratense*, *Dactylis glomerata*, *Trifolium pratense*, *T. hybridum*, *T. repens*, *Vicia sativa*, *Pisum sativum*, *Linum usitatissimum*, *Solanum tuberosum*, *Galeopsis pubescens* and *G. speciosa*. The results were in most cases negative, but in three species, *Linum usitatissimum*, *Solanum tuberosum* and *Galeopsis pubescens*, chromosome doubling was obtained. These positive results may justify a brief contribution to the rapidly increasing colchicine literature.

In all the species, except *Galeopsis*, the treatment was given to the resting seeds. In most cases the seeds were submersed in weak colchicine solutions (0,05 or 0,025 per cent.) for 3—6 days. In the two *Galeopsis* species the treatment was given to the seedlings. The most convenient method in this case was to use a mixture of 1 per cent. colchicine in lanoline, as recommended by BLAKESLEE and AVERY (1937).

The seeds treated with colchicine had a more or less reduced germination, and many of the seedlings obtained presented the characteristic colchicine abnormalities. Strikingly abnormal seedlings were especially obtained in wheat and barley, and undoubtedly these seedlings were largely composed of tissues with an increased chromosome number. Gradually, however, these deviating sectors were replaced by normal tissue, and when the plants had reached the flowering stage all the ears were found to be identical in appearance with the untreated control plants and, consequently, also the chromosome number was certainly unchanged.

In *Linum* and *Solanum*, however, the seed treatment was more successful. In flax two different seed portions treated with a 0,025 per cent. solution for 2 and 4 days respectively gave a total of 6 plants with doubled chromosome number. Four plants of this kind, resul-

ting from the shorter treatment, were found to have the chromosome numbers ± 58, ± 59, ± 60 and ± 61. (For the chromosome counts reported in this paper we are indebted to Miss E. HOLMQVIST.) In the two other tetraploids, obtained after a four-day treatment, the number of chromosomes was ± 59 and ± 60 respectively. Though it was difficult to make quite exact counts, it is probable that there were really some slight differences in the chromosome numbers of these plants. Tetraploid flax has already been produced by SIMONET, CHOPINET and SOUILIJAERT (1938), GYÖRFFY (1938) and LUTKOV (1938), but, apparently, the exact chromosome numbers of the tetraploids produced by these authors have not been determined. This may partly be due to the smallness of the flax chromosomes, partly to slight numerical variation. In our own material (a variety of oil flax) the diploid control plants also seem to have somewhat different chromosome numbers. In five plants studied the counts gave the following results: $2n = 32$ (2 plants), $2n = \pm 32$ (1 plant), $2n = 31$ (1 plant) and $2n = \pm 31$ (1 plant).

From the primary tetraploids some progenies were raised. In one of these the chromosome numbers of four plants were found to be ± 62 (2 plants), 61 (1 plant) and ± 61 (1 plant). Slight chromosomal variation in the offspring of an autotetraploid may, indeed, be expected, but the results evidently indicate strongly that there is also a chromosomal variation among the primary tetraploids and even in the diploid control material.

In contrast to flax the other two tetraploids produced by us are novelties not previously induced by colchicine treatment. The potato tetraploids were obtained by treating seeds of the commercial variety »Triumph» in a 0,05 per cent. solution for 6 days. Some of the plants obtained were morphologically rather deviating and showed all signs of having a doubled chromosome number. Measurements of stomata supported this impression, the stomata in the supposed tetraploids being significantly larger than in the normal control plants.

Good root tip fixations were obtained in the following spring from several clone plants. These counts demonstrated that two of the individuals from colchicine treated seeds had exactly 96 chromosomes. Of eight chromosome plates examined, seven gave the number 96 and only in one plate the number seemed to be 97.

In addition to these plants with 96 chromosomes a third individual, much to our surprise, was found to have the chromosome number ± 64 (two plates with 64, one with 63 chromosomes). Thus, in this

case the effect of the colchicine was the production of a cell and probably a whole plant with 16 chromosomes more than the normal number 48. As might be expected from its aneuploid chromosome number, this plant has a rather poor vigour.

As already mentioned, the tetraploids of *Galeopsis pubescens* were obtained by treating seedlings with 1 per cent. colchicine in lanoline. Three plants, belonging to three different pure lines (P—A, P—B and P—C), were observed to have large flowers. The P—A and P—B plants in question were rather well developed and had normal anthers, allowing successful self-pollination. The P—C plant, however, was very weak, had abnormal anthers and was therefore pollinated with

TABLE 1. *Pollen size after colchicine treatment of Galeopsis pubescens.*

Line	Pollen diameter							n	M	Chromosome number
	13	14	15	16	17	18	19			
P—A, colchicine.....			4	15	50	26	5	100	17,1	32
» , control	41	52	7					100	13,7	16
P—B, colchicine				7	38	43	12	100	17,6	32
» , control	6	69	25					100	14,2	16
P—A, field plant		26	69	5				100	14,8	»
P—B, »	1	53	46					100	14,5	»
P—C, »		40	59					99	14,6	»
T—B, »			3	11	63	23		100	17,1	32

pollen from the P—A plant. This resulted in a few seeds and some hybrid plants, now growing in the experimental field.

Some offspring of the large-flowered P—A and P—B plants were raised in the winter under electric light. Chromosome counts in root tips were undertaken in three descendants of the P—A plant and in two daughter plants of the P—B individual. All these plants were found to be exactly tetraploid, having $2n = 32$ instead of the normal number $2n = 16$, found in the control plants. — This result was not unexpected, inasmuch as the mother plants differed from the controls not only in flower dimensions but also in pollen size. The results of the pollen measurements are given in Table 1.

The diploid types in the table are represented by green-house plants of P—A and P—B, field plants of the same lines and also a field plant of the P—C line. The average values of these plants are very similar and vary from 13,7 to 14,8 units. The mean values of the

relatively weak pot-plants in the greenhouse are only slightly lower than the corresponding values of the field plants.

According to the table, the tetraploids have much larger pollen, the average values ranging from 17,1 to 17,6 units. It is interesting to note that a pollen sample of the natural tetraploid species *Tetrahit* (T—B) was found to give just the same average size as the tetraploid *pubescens* plants. The significance of the size differences between the diploid and tetraploid pollen is at once evident from the entirely different distributions of the variates, and therefore the standard errors were not calculated.

Though pollen could not be obtained from the large-flowered P—C plant, it is highly probable that this plant was also tetraploid. In *Galeopsis* crosses between diploids and tetraploids always fail. As the plant in question gave offspring after pollination with the tetraploid P—A plant, it must have been tetraploid. Thus, the colchicine experiments with *G. pubescens* evidently resulted in two autotetraploid lines and in some tetraploid hybrids between the lines P—A and P—C.

It may be mentioned that in colchicine experiments carried on by the senior author this summer tetraploidy has probably also been induced in *Galeopsis speciosa.* A number of large-flowered shoots were all found to have large pollen grains and the difference between large and normal pollen in this species seems to be just as clear as in *G. pubescens.* Since evidence has been obtained (MÜNTZING, 1930, 1932) that the tetraploid *Galeopsis Tetrahit* is derived from the diploid species *pubescens* and *speciosa*, the production of tetraploid *pubescens* and *speciosa* strains is quite important for further studies on the relationship between the diploid and tetraploid *Galeopsis* species in question.

The chromosome doubling in the diploid species is also important for the realisation of new cross combinations. Thus, for instance, ordinary diploid *pubescens* does not give any hybrids when crossed with *Tetrahit*, but tetraploid *pubescens* could be successfully crossed with *Tetrahit*, and a number of F_1 hybrids are now grown in the field. Thus the colchicine experiments in *Galeopsis*, as in many other plant species, have resulted in a new and interesting research material, which in the present case will also facilitate new attacks on old problems.

Besides the production of polyploids in some species, another effect of the colchicine treatment in our material was rather striking. Especially in some of the grass species (*Festuca pratensis* and *Lolium perenne*) the plants obtained from treated seeds were much more vigorous than the control plants. In several cases this vigour was so

marked that the plants were suspected to be polyploids. Chromosome counts, however, showed the chromosome number to be unchanged, and thus the only conclusion to be drawn is that the treatment of the seed with weak colchicine solutions may have a stimulating effect on the growth of the seedlings. This stimulating effect was most marked at an early stage and seemed to gradually disappear. These observations agree very well with the results of HAVAS (1938). In various plant physiological experiments with very weak colchicine solutions this author found a growth-stimulating effect. This effect is thought to be due to the action of colchicine on the plant hormones present and not to a direct effect of the colchicine itself.

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